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Volume I

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March 1976

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TOXICOLOGY OF METALS - Volume I

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16. ABSTRACT

This report consists of three main sections. The first (36 p.) is the transcript of a panel discussion of a meeting of the Subcommittee on the Toxicology of Metals. It covers the general principles and mechanisms of absorption and excretion of all metals, but of heavy metals in particular. The second section, by a task group from the Subcommittee, consists of additional information on accumulation and retention ot toxic metals, with special emphasis on absorption, excretion, and biological half-times, particularly of cadmium, lead, and mercury. The third section (182 p.), a consensus report of a Subcommittee meeting, covers the dose-effects and dose-response relationships of toxic metals, specifically cadmium, lead, and mercury. The second and third sections contain information on critical organs, effects, and concentrations, as well as on models of retention and excretion and homeostatic mechanisms in general.

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TOXICOLOGY OF METALS - Volume I

By
Subcommittee on the Toxicology of Metals
Permanent Commission and International Association of
Occupational Health
Prof. Lars Friberg, Chairman

in cooperation with

The Swedish Environmental Protection Board, and
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LIST OF ABBREVIATIONS
(Used in Section IV)

ALA-δ–amino levulinic acid
ALA-D-δ–amino levulinic acid dehydrase
ALA-S-δ–amino levulinic acid synthetase
ALA-U-δ–amino levulinic acid in urine
AMD–aerodynamic mass diameter
cAMP–adenosine-3':5'-cyc-monophosphate (Cyc 3':5'-AMP)
CNS–central nervous system
CPG–coproporphyrinogen
CP-U–coproporphyrin in urine
EDTA–ethylenediamine tetraacetic acid
FEP–free erythrocyte protoporphyrin IX or fluorescent porphyrin
Heme-S–heme synthetase
ICMACMC–International Committee on Maximum Allowable Concentrations of Mercury Compounds
L-chains–light chains, example: gamma globulin L-chains
LD$_{50}$–lethal concentration for 50% of a population
MeHg$^+$–methyl mercuric radical = CH$_3$Hg$^+$
MMAD–mass median aerodynamic diameter
m.w.–molecular weight
Pb-B–lead in blood
Pb-U–lead in urine
PAH–paraaminohippurate
PBG–proporphobilinogen
PP–protoporphyrin (always followed by a Roman numeral which designates the type of protoporphyrin)
RBC–red blood cells
RBP–retinol binding protein
TGMA–see Task Group on Metal Accumulation in Section IV. 8
-SH group–sulfhydryl group
1,25-OH-cholecalciferol–a vitamin D metabolite
w.w.–wet weight

SECTION I

INTRODUCTION

SECTION I
INTRODUCTION

At the time of the XVIth International Congress on Occupational Health in Tokyo in 1969 the Permanent Commission and International Association of Occupational Health decided to form a subcommittee on the toxicology of metals. The need for such a group had been recognized during an international meeting on mercury toxicology in 1968. The subcommittee was formed with Prof. Lars Friberg of Stockholm as chairman. The program developed by this group consisted of exploration and documentation of the general principles of metal toxicology by means of symposia and workshops of experts in the field. A broad approach was selected including general environmental exposures as well as those related to occupation. Emphasis was placed on greater depth and precision of understanding in terms of metabolic processes and dose-effect relationships. The Permanent Commission, the Swedish Environmental Protection Board, and the Karolinska Institute have supported the committee in carrying out this program. Two workshops were conducted, one in conjunction with the meeting of the Permanent Commission in 1971, and another in conjunction with the XVIIth International Congress on Occupation Health in 1972.

Recently the subcommittee has decided to broaden its work by preparing a handbook on metal toxicology which will contain the general material being developed in its workshops and also specific information on a large number of metals for use by toxicologists and occupational and environmental health workers. The Environmental Protection Agency is cooperating with the other sponsors in supporting this phase of the subcommittee's work. As information is developed on the subject it will be collected in three annual progress reports. The first of these is contained in this volume. It consists of the conclusions of the subcommittee's third workshop which was held in Tokyo in 1974.

Since the three workshops which have been held constitute a cumulative series, with frequent reference back to previous definitions and statements, it was thought best on this occasion to reproduce all of them together as a unit. Therefore, the body of this report contains three sections, the first two being reproductions of the already published conclusions of the first two workshops, and the third being the additional material produced last fall

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at the meeting in Japan. We are grateful to the scientists and institutions concerned, and also to the journals, Nordisk Hygienisk Tidskrift and Environmental Physiology and Biochemistry, for permission to reproduce these earlier publications. We are grateful, as well, to Elsevier Scientific Publishing Company for permission to preprint the information that comprises Section IV, which, along with working papers from the meeting, they will publish subsequently.

SECTION II

ABSORPTION AND EXCRETION OF TOXIC METALS

Report from a panel discussion at the meeting
of the Subcommittee on the Toxicology of Metals held in
Slanchev Briag, Bulgaria, September 24, 1971.

Edited by

Kersti Dukes, B.A. and Lars Friberg, M.D.

(Reprinted from Nordisk Hygienisk Tidskrift,
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SECTION II

ABSORPTION AND EXCRETION OF TOXIC METALS

Report from a panel discussion at the meeting of the
Subcommittee on the Toxicology of Metals, *)
held in Slanchev Briag, Bulgaria, September 24, 1971.

edited by
Kersti Dukes, B.A. and Lars Friberg, M.D.

II.1. BACKGROUND

At the meeting of the Permanent Commission and International Association on Occupational Health in Slanchev Briag, Bulgaria, September 20—24, 1971 the various subcommittees had been asked to arrange their own programs. The Subcommittee on the Toxicology of Metals had chosen as its main theme "Metal Absorption and Toxicity by Oral, Inhalatory and Cutaneous Routes". The last session of its meetings was devoted to a panel discussion aimed at summing up the general principles and basic data on absorption, excretion and biological half-lives of toxic metals.

In the panel participated Lars Friberg, M.D., The Karolinska Institute, Stockholm, Sweden (Chairman), Maths Berlin, M.D., The University of Lund, Lund, Sweden, Thomas Clarkson, Ph.D., The University of Rochester, Rochester, New York, USA, Lennart Danielson, Ph.D., Environment Protection Board, Stockholm, Sweden, Robert Goyer, M.D., The University of North Carolina, Chapel Hill, N.C., USA, Norton Nelson, M.D., New York University Medical Center, New York, N.Y., USA, Magnus Piscator, M.D., The Karolinska Institute, Stockholm, Sweden, Kenzaburo Tsuchiya, M.D., Keio University, Tokyo Japan, and Jan Wahlberg, M.D., The Karolinska Institute, Stockholm, Sweden.

From the audience participated the following persons, Sven Yllner, M.D., Swedish Employers Association, Stockholm, Sweden, Tadeush Dutkiewicz, M.D., Medical Academy of Lodz, Poland, Tor Norseth, M.D., Institute of Occupational Health, Oslo, Norway, Miroslav Cikrt, M.D., Institute of Hygiene and Epidemiology, Prague, Czechoslovakia, Dennis Malcolm, M.D., Wilmslow, England, George P. Lewis, M.D., Veterans Administration Hospital, Boston, Mass., USA.

The chairman opened the session and it was decided to consider first,

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*) The meeting was financially sponsored by the Permanent Commission and International Association of Occupational Health and the Swedish Environment Protection Board.

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questions on absorption of metals and thereafter questions on excretion and biological half-lives. Dr. Nelson was asked to start out with absorption via inhalation.

II.2. PANEL DISCUSSION

NELSON: I believe that absorption and uptake by inhalation are presently capable of more reliable generalizations than uptake by the cutaneous route or gastro-intestinal absorption. I say this because I believe the system can be described more accurately in mechanical terms and with fewer biological variants than is possible with the intestinal or the cutaneous system. Basically, in the pulmonary tract we are dealing with a relatively straightforward system for the uptake of inhaled materials.

For the purpose of the present discussion, I will use the term "absorption" to apply to that portion of the inhaled substance which has entered or crossed one of the surface membranes of the respiratory tract; under this definition the subsequent fate of the substance is irrelevant to its "absorption".

The major systemic absorption of inhaled materials occurs in the deep pulmonary tract, the alveolar part of the lung, which has a very thin diffusible membrane with apparently relatively few selective properties. This contrasts with the highly selective system found especially in the intestinal tract and perhaps to a lesser extent in the cutaneous system. Accordingly, I am going to review those patterns of inhalation and deposition which are the basis for respiratory absorption.

The patterns of respiratory deposition have undergone extensive study over the last 30 years. It is one of the triumphs of the physiological modeling that a prediction, developed by Findeisen, 1935, on the characteristic patterns of respiratory deposition, has been confirmed in general and indeed in some detailed features.

Findeisen analyzed the deposition from purely engineering and mechanical standpoints in which he defined the several respiratory segments, developed patterns of airflow, taking into account the three major means by which dust reaches the respiratory surfaces. These three are, inertia with the very large particles, settling or gravity with the large and intermediate particles and Brownian motion (molecular bombardment) with very fine particles.

Findeisen's predications have now been verified in several studies in humans, including work in our own laboratories (Altshuler, Yarmus and Palmes, 1957). The general pattern is that the total deposition is high at the large particle sizes and falls through a minimum at around 0.4 microns for unit density particles. It tends to rise again with smaller particles where their mobility permits them to be moved by the molecular movement of the gases. It has become a dictum of this particular field that a "hit"

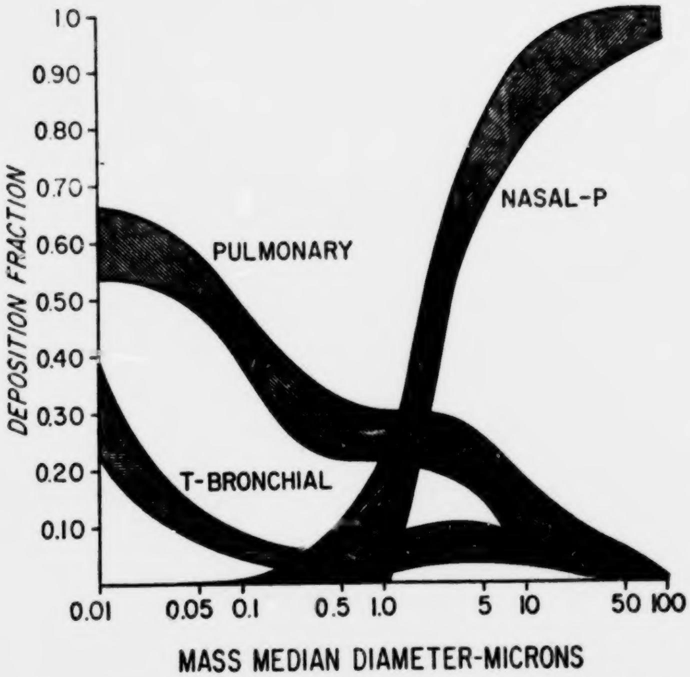


Figure 1. Patterns of Particle Deposition in Various Portions of the Respiratory System (from Task Group on Lung Dynamics, 1966).

is a "trap" meaning that a particle once reaching the respiratory surface is there trapped and is no longer airborne.

The subsequent fate of particles is then determined by what portion of the lung that they are deposited in and the chemical and physical characteristics of the particle.

There is a critical size dependency of particle deposition within the respiratory tract. The large particles are mostly filtered out in the nasal passages while there is heavy deposition in the deep pulmonary system of particles in the one to two micron range and again in the fractional micron sizes, see Figure 1.

Generally speaking, relatively insoluble particles, which are deposited in the upper airways, those with ciliated surfaces, will be moved on up via the mucous flow under the ciliary motion. They will then be either ex-

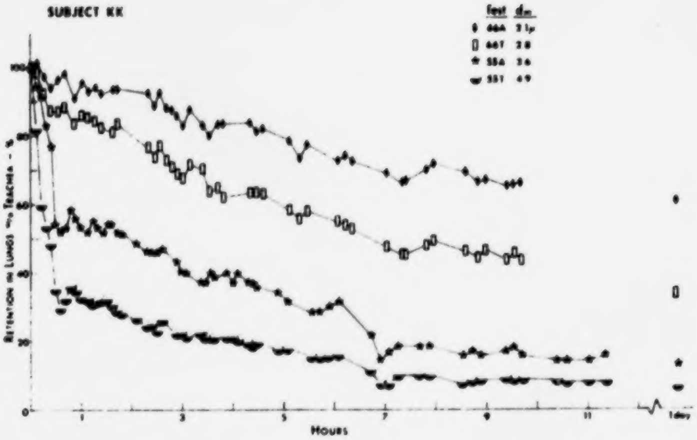


Figure 2. Clearance Curves of Iron Oxide Particles from the Lungs of a 21 Year Male (from Albert, Lippmann and Briscoe, 1969).

pectorated or swallowed and thereby subject to gastro-intestinal absorption depending on their chemical characteristics.

On the other hand those materials that reach the deeper lungs have several fates in store for them. One is that they may be immediately dissolved and distributed systemically, or they may be phagocytized and enter the parenchyma of the lung. The fate of phagocytized particles again depends on the physical and chemical properties which will alter their residence time and determine the extent of their contribution to body burden. Both extent and locus of deposition are subject to a variety of factors; the respiratory rate and volume are particularly important.

Figure 2 illustrates a series of more recent examinations of particle behavior in humans carried out at our Institute based on the use of spherical iron oxide particles (Albert, Lippmann and Briscoe, 1969). The technique, which measures both deposition and clearance, is based on the inhalation of monodisperse particles so generated that they will be uniform in particle size over a range of sizes. The particles are spiked with a radionuclide and the rate of clearance is measured externally by a scintillation detector. As shown in Figure 2, the total amount deposited in the deep lung, which corresponds to the slow portion of the clearance curve, is least in the 4.9 microns particle size and greatest in the smallest particle size. The initial clearance rates (at the left hand side of the figure) which are very rapid correspond to the ciliary clearance patterns and show how rapidly the particles are mobilized from the ciliated airways. The slow phase (to the right in the figure) represents clearance of particles deposited in the deeper airways. The rate of migration from the lower airways is a complex one—partially phagocytosis and subsequent mobilization into the ciliary tract, partially passing into the lung parenchyma with slow disappearance from that region.

One can use techniques of this sort to estimate the amount of material entering the deep lung where it is most readily absorbed and where it will be most available for contributing to the systemic body burden. These techniques could be extended to materials other than iron dusts used in these particular experiments. Thus, I think we do have tools for a systematic examination of the rate of uptake of inhaled metals and other materials.

The employment of the procedures I have described represents a relatively sophisticated and so far, little used approach. Meanwhile a cruder and more generalized technique for estimating respiratory absorption has been published by the International Commission on Radiologic Protection (Task Group on Lung Dynamics, 1966). They characterize particles according to three classes of solubility. The classes correspond roughly to the half-times of solubilization in the lung, one day, one week and one year. Using this model it is mathematically possible to make crude estimates of body uptake and absorption.

CHAIRMAN: Is it possible to predict in a systematic way how different metals are absorbed from the lungs into the body, e.g. cadmium versus lead versus mercury versus manganese?

NELSON: One very direct way to do it is in tracer studies through the use of radio tags in which one can measure the compound in the appropriate organ, be it blood or other tissue. One can also use the classical techniques of examining distribution and excretion.

CHAIRMAN: Are such data lacking for the time being? My own feeling is that it is not possible to give data for different metals and particle sizes whether the absorption rate is e.g. 10 percent, 25 percent or 40 percent.

NELSON: Yes, mostly they are lacking. The ICRP approach does give a basis for very crude estimates. To refine this, one should go to the actual measurement of specific materials; this is a quite feasible way.

The chairman then asked Dr. Clarkson to proceed with absorption via the intestinal tract.

CLARKSON: You will notice the marked contrast between the rather sophisticated, well-studied situation in the respiratory tract versus the remarkable lack of data on gastro-intestinal (G.I.) absorption. I should like

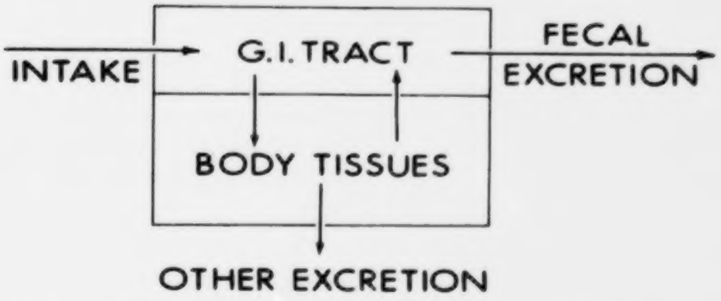


Figure 3. A Compartmental Model describing the Input-output Relationship during the Absorption of Metals from Food.

to summarize the results of previous attempts at the measurements of gastro-intestinal absorption of metals in human subjects. Figure 3 simply defines diagramatically the input-output relationship with regard to the ingestion of metals with food. The G.I. tract is represented by the upper compartment. Metals absorbed from the G.I. tract enter the lower compartment. Excretion is assumed to be by way of urine and via feces. The real in vivo situation is obviously a great deal more complicated than this. For example methyl mercury compounds are probably absorbed in the stomach, excreted in the bile and reabsorbed again in the lower intestine. However, in this diagram, the two vertical arrows represent G.I. absorption and excretion and must be defined in terms permitting experimental measurements.

In their attempts to measure these processes previous workers have made the following explicit or implicit definitions. The G.I. absorption is the amount of metal absorbed from food when the body burden is zero. G.I. excretion is equal to the fecal excretion when the intake of metal in food is zero. Thus, the two arrows depicted in this diagram for absorption and excretion in the G.I. tract do not take into account the enterohepatic recirculation where the excretion of the metal in the bile is cancelled by the subsequent reabsorption in the lower intestine.

G.I. absorption cannot usually be directly measured in human subjects. For example, the administration of an oral dose of a metal labelled with a gamma-emitting isotope will permit measurements of retention by whole body counting techniques. The whole body count will include of course the radioactivity of unabsorbed metal in the G.I. tract and correction must be made for this. Furthermore, the whole body count will decline due to excretion and we need to correct for this also. Nevertheless, whole body counting methods have been used successfully in the case of compounds

Table 1. Lead Intake and Output of a Normal Subject During a Two Year Period of Oral Administration of Lead (from Kehoe et al., 1943).

Ingested	percent total
In drinking water	0.8
In food and beverages	8.5
Administered in solution	90.7
Total	100.0
Excreted	
In feces	87.1
In urine	5.1
Total	92.2
Retained	7.8

of mercury and cadmium. Miettinen, 1971, in press, has reviewed observations on volunteers given single doses of compounds of methyl mercury and inorganic mercury. Similar studies on methyl mercury by Aberg et al., 1969, have also been reported. The metal has been administered in food, bound to fish or liver protein or in aqueous solution. Mercury was labelled with the gamma-emitting isotope ^{203}Hg having a half-time of 47 days. Whole body counts were made at least daily for the first few days. A graph was plotted on semilogarithmic paper for whole body counts against time.

In the study on inorganic mercury the whole body count declined rapidly during the first two or three days corresponding to fecal excretion of unabsorbed mercury. Subsequently the whole body activity declined at a much slower rate equivalent to a biological half-time of approximately 50 days. The intercept obtained by extrapolating the second line back to zero time gave the amount of oral dose absorbed. A similar procedure was followed for methyl mercury and cadmium salts. In the case of all these compounds, excretion of the metal was very slow, allowing the rapid decline in the whole body count due to clearance of unabsorbed metal to be distinguished from the much slower decline due to excretion of the absorbed metal.

Miettinen, 1971, in press, has reported the following values for absorption from single oral doses. Approximately 15 percent inorganic di-valent mercury is absorbed from food. Methyl mercury compounds are absorbed to 90—100 percent and cadmium salts to approximately 5 percent.

Lead does not have a gamma-emitting isotope suitable for whole body counting techniques. It does, however, offer the advantage that the gastro-intestinal excretion is negligible. Two experimental approaches have been taken to the measurements of the gastro-intestinal absorption of lead in

Table 2. Absorption of Orally Administered ^{212}Pb calculated by comparing the Urinary Excretion with that after Intravenous Administration (from Hursh and Suomela, 1968).

Subject	Dose μc	Mode	24 hr urinary loss μc	Absorption μc	Absorption % dose	48 hr fecal excretion μc
A	1.08	i.v.	.053			.003
D	1.17	i.v.	.040			.003
A	5.05	oral	.003	.067	1.3	
B	5.02	oral	.017	.404	8.1	
C	4.80	oral	.032	.768	26.0	

humans. Kehoe et al., 1943, reported on the results of lead balance in individuals given lead in food for a period of two years. Table 1 gives the results on one subject taking 2 mg of lead salts for two years. Essentially, a balance study was carried out in which the daily intake of lead from food, beverages and water was compared to the fecal and urinary excretion. Kehoe et al., 1943, have presented evidence that the clearance of unabsorbed lead from the gastro-intestinal tract accounts for all of the fecal excretion. Measurements of fecal excretion of lead following cessation of chronic oral doses revealed that within a few days fecal lead excretion was indistinguishable from fecal excretion prior to exposure despite the fact that the body burden was still elevated. Hursh and Suomela, 1968, demonstrated that fecal excretion of lead was very low as compared to urinary excretion in two individuals receiving an intravenous dose of ^{212}Pb as $PbCl_2$ (Table 2). Thus, in the balance studies reported by Kehoe et al., 1943, the amount retained over two years plus the amount excreted in the urine will be equal to the amount absorbed. The amount absorbed, according to Table 1, was approximately 13 % of the ingested lead.

Hursh and Suomela, 1968, reported observations on human volunteers given lead salts labelled with the isotope 212. The half-time of this gamma-emitting isotope, 10.6 hours, is too small to permit measurement of absorption by whole body counting techniques. It will take too long, 70 hours or more (about 7 half-times), before the G.I. tract is cleared of unabsorbed lead. Hursh and his colleagues made the assumption that the amount of radioactive lead excreted in urine for the first 24 hours was proportional to the amount of absorbed lead in the body. They gave a known amount of radioactive lead chloride intravenously to two subjects and measured the first 24 hours of urinary excretion, which was 5 percent of the injected dose (Table 2). They gave 3 other subjects an oral dose in beer (lead-212 is generated from radon and the gases trapped in beer) and also measured

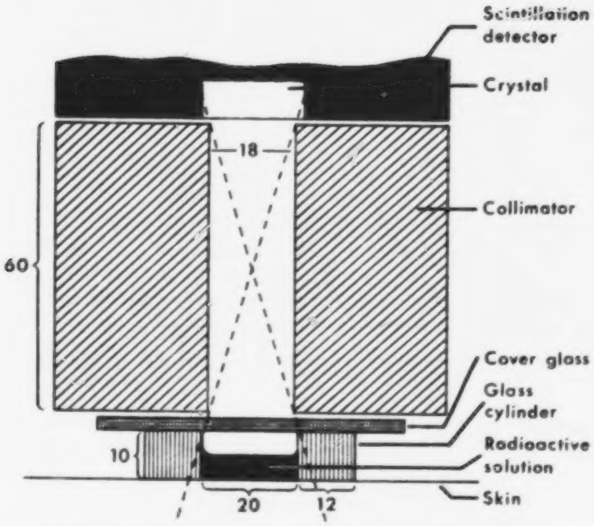


Figure 4. Schematic Presentation of the Arrangement for Disappearance Experiments in vivo. Unit of Measurement: Millimeter (from Wahlberg, 1965 a).

urinary excretion over the first 24 hours. The amount of lead absorbed in the first 24 hours was calculated from the relationship:

$$\text{Amount of oral dose absorbed} = \frac{\text{24 hr. urinary lead (oral dose)} \times \text{(i.v. dose)}}{\text{24 hr. urinary lead (i.v. dose)}}$$

The values obtained on three subjects ranged from 1.3 percent to 16 percent (Table 2).

Factors influencing gastro-intestinal absorption of metals are poorly understood. Miler, Sattler and Menden, 1970, have shown that the retention of ^{212}Pb may be increased by 7 to 49 % by increasing the protein content of isocaloric diets of experimental animals. On the other hand, methyl mercury compounds undergo virtually complete G.I. absorption whether given as the chloride salt or complexed with fish protein (Åberg et al., 1969, Miettinen, 1971, in press). Friberg, Piscator and Nordberg 1971, have reviewed literature reports indicating that low dietary calcium leads to enhanced absorption of cadmium in experimental animals. One explanation offered is that low dietary calcium slows the passage of cadmium along the digestive tract. The rate of transit along the G.I. tract may be an important consideration for all metals that undergo slow absorption.

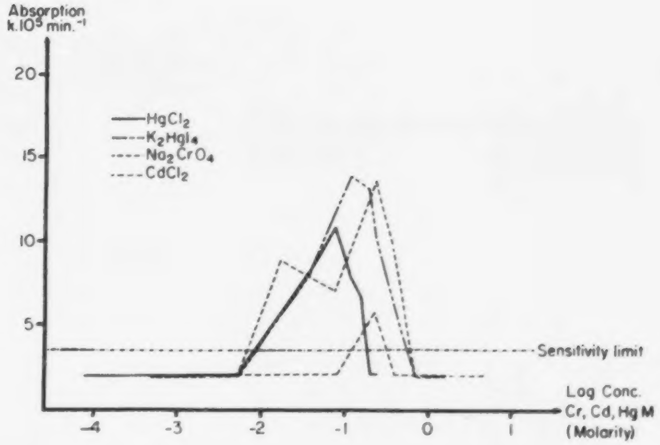


Figure 5. The Relative Absorption at Different Concentrations of Sodium Chromate, Cadmium Chloride, Mercuric Chloride and Potassium Mercuric Iodide (mean) (from Skog and Wahlberg, 1964).

The chairman then asked Dr. Wahlberg to continue with skin absorption.

WAHLBERG: Percutaneous absorption is defined as the penetration of substances from the outside into the skin and thereafter through the skin and into the blood and lymph vessels. Percutaneous toxicity is defined as the systemic poisoning following penetration of toxic materials through the cutaneous barriers and their distribution throughout the whole body.

Figure 4 shows a schematic arrangement for the disappearance measurements in vivo, where the radioactivity above a deposit of an isotope labelled metal on the skin is continuously recorded by a scintillation detector. The decrease in radioactivity is an indication of absorption. The mean relative absorption for different substances and at different concentrations is seen in Figure 5. It increases with increasing concentrations up to a maximum which seems to be characteristic for each substance. If the concentration is increased still further, the relative absorption decreases gradually. In Figure 6 the absolute absorption is correlated to the applied amount. At lower concentrations the absorption increases with rising concentrations. However, despite further increase in concentration and hence also an increase in the total amount applied, absorption does not continue to rise,

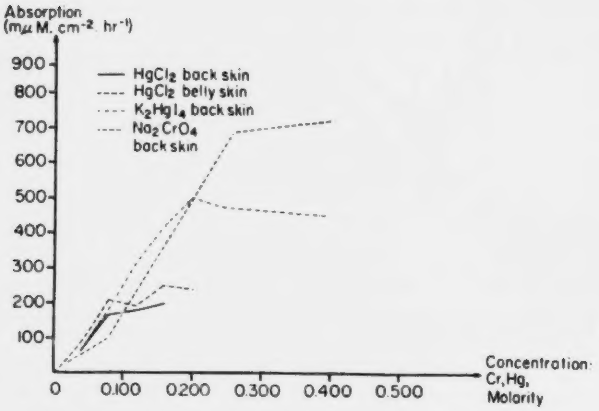


Figure 6. The Absolute Absorption of Sodium Chromate, Mercuric Chloride and Potassium Mercuric Iodide (mean) correlated to the Applied Amounts (from Skog and Wahlberg, 1964).

but a level is reached for sodium chromate and potassium mercuric iodide.

The absorption conditions for 12 metal compounds are summarized in Table 3. Relative absorption varies from less than 1 percent to 4.5 percent during a 5 hour period.

Table 3. Mean Relative Absorption from Aqueous Solutions of 12 Metal Compounds. Exposure Area: 3.1 cm².

Compound	Isotope	Relative Absorption percent / 5 h
$Na_2Cr_2O_7$	^{51}Cr	<1.0
$CoCl_2$	^{58}Co	<1.0
$ZnCl_2$	^{65}Zn	<1.0
$AgNO_3$	^{110m}Ag	<1.0
$CdCl_2$	^{115m}Cd	1.8
$CrCl_3$	^{51}Cr	2.2
NaCl	^{22}Na	2.9
$SrCl_2$	^{89}Sr	3.1
$HgCl_2$	^{203}Hg	3.2
Na_2CrO_4	^{51}Cr	4.0
K_2HgI_4	^{203}Hg	4.1
$CH_3HgC_2H_3N_4$	^{203}Hg	4.5

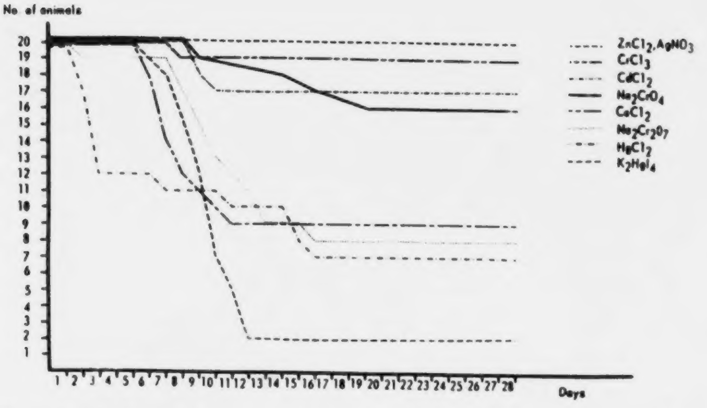


Figure 7. Number of Surviving Animals at Different Times after Percutaneous Application of Metal Compounds (2.0 ml of 0.239 M Aqueous Solutions) (from Wahlberg, 1965 b).

The differences in absorption rate cannot solely explain the variations in the potency to induce hypersensitivity. Cobaltous chloride and bichromate, which are well-known sensitizers, have comparatively low absorption rates.

In the percutaneous toxicity tests 2 ml of an aqueous solution of a metal compound were applied. The skin depot had the same exposure area as in previous absorption experiments, i.e. 3.1 cm^2. The mortality increased with increasing concentrations and most of the animals died between the 7th and the 15th day. The final comparison among nine metal compounds was made at a concentration of 0.239 molar. Figure 7 shows that all animals exposed to zinc chloride and silver nitrate survived, whereas only 2 animals exposed to potassium mercuric iodide survived. With few exceptions there was a good agreement between observed and expected mortality on the basis of known absorption rates and intraperitoneal toxicity. The animals exposed to chromic chloride manifested a parallel weight increase with that of animals exposed to distilled water and controls, whereas the mean weight increase for animals treated with zinc chloride and silver nitrate ceased by the first week. When a small amount of mercuric chloride was applied, the mean weight decreased during the first week of the observation period.

CHAIRMAN: These three presentations are now open for discussion and

I invite both members of the panel and the audience to take part in the discussion.

CLARKSON: This is a question to Dr. Goyer about the fecal excretion of lead. Data reported in the literature indicate that fecal excretion is very small, but, in an earlier session, you presented evidence that lead is found in the bile. Is there an enterohepatic recirculation of lead as observed in the case of methyl mercury?

GOYER: I presume it is very similar. The data I presented about lead being excreted in bile were from a work by Castellino and Aloj, 1964, in which they injected labelled lead and then recovered about 80 percent of the label in bile. Similar studies have been done by Black, 1962, with dogs. When talking about an absorption of lead with regard to a value for the gastro-intestinal absorption, we are really talking about a *net* absorption of 10—15 percent.

I would like to make one further comment with regard to this. We are talking about people without excessive body burden of metals and I am not sure that we can extrapolate this degree of absorption truthfully in persons who have greater or increased body burdens. From our studies in rats fed lead from 80 ppm to 10,000 ppm, we had a variation of absorption from 1—2 percent with a minimal dose, i.e. 80 ppm to a very small fraction of one percent ($<0.1\ \%$) with an oral intake of about 10,000 ppm lead (Goyer et al., 1970). These numbers should not be translated directly to what would apply to man, but I think that the principle can be applied, that is, if we have someone with excessive exposure, the efficiency of absorption may not be expected to equal the 10 to 15 percent that we would see in a non-exposed or slightly exposed person. These kinds of data, that is, with several times usual environmental exposure, are not available from human observations. I think it is difficult to extrapolate what we know about non-exposed people to heavily exposed people.

CLARKSON: In Kehoe's study the subjects were given 2 mg of lead a day for 2 years, thus giving a substantial body burden. Absorption from the gastro-intestinal tract was 13 percent. The evidence that there is a small fecal excretion of lead is from Kehoe's work where subjects would inhale lead and increase their body burden in this way. Although the urinary excretion increased in parallel to the retention, there is very little change in the fecal excretion. Once the subjects were taken off exposure and when the unabsorbed lead had been cleared from the gastro-intestinal tract, the fecal excretion hardly changed at all, even though their body burden and urinary excretion had greatly increased. Hursh and Suomela, 1968, studying much lower doses of lead in human volunteers came to the same conclusion. In their study, fecal excretion of lead was measured in individuals given a single intravenous dose of ^{212}Pb in the form of $PbCl_2$.

BERLIN: One important question concerning absorption via inhalation is to what extent the general model can be adapted to humans in real life in industry. There is a number of factors that should be taken into consideration. Liquids of different kinds easily penetrate down to the peripheral part of the lung. Irritating gases often accompany metal fumes or particles and may give rise to effects, e.g. secretion of mucous and bronchial construction. Mucous secretion may occur to such an extent that reflux of secretion in the bronchial tree and even down to the peripheral part of the lung may occur and thus change the model concerning retention and absorption considerably.

NELSON: I accept totally the suggestion that in "real life", as found in community air pollution or occupational exposures, the situation is different from that in carefully controlled laboratory studies. The latter are different in a variety of ways; among others, the particle size is known and uniform. This is a pretty rare circumstance in a natural situation. What I did not go into at all are the physiological variables that alter host responses. For example, clearance patterns are very much subject to a whole series of physiological factors including disease and presence of irritants. Such factors will influence patterns of respiration and patterns of clearance. I have presented only general principles; where they have been applied, even in a crude way, for example to airborne lead experimentally, these principles were verified within expected limits.

DANIELSON: I have a general question to all the experts here on absorption. To what extent is absorption a passive or an active (e.g. energy requiring) process? This question may have some bearing on what was mentioned today by Dr. Goyer, that the more lead you ingest, the less percentage you absorb. If this reflects a toxic effect on an active absorption process, it is possible that other compounds, which e.g. are absorbed from the gastro-intestinal tract, behave in the same way.

GOYER: Bingham et al., 1968, studied the effect of continued inhalation of small particle size (99 % less than $0.1\ \mu$ in diameter) of lead and found that the number of alveolar macrophages actually cleared from the lung was decreased. It is interesting to speculate what influence this finding has on the ability of the lung to absorb and excrete lead. The amount of lead being excreted with the alveolar cells has not been determined. This type of study perhaps represents the level where further work should be done.

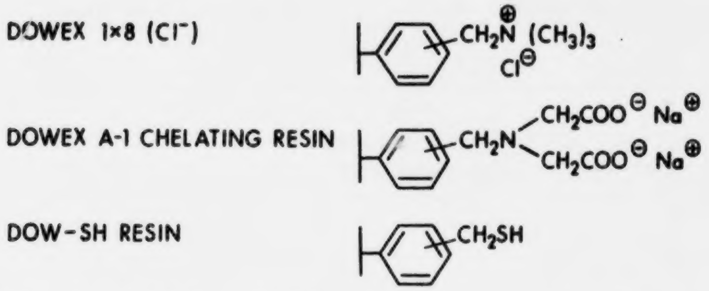


Figure 8. Chemical Structures of Resins Added to Food to Test Reduction of Gastro-Intestinal Absorption of Mercury (from Clarkson, Small and Norseth, 1971).

I think a lot of the clearance studies done today give us information about deposition in the alveolar cells, but I do not think they tell us very clearly how much really gets into the blood.

YLLNER: In view of recent knowledge on absorption could you comment upon the old question of giving, for example lead workers, certain amounts of milk every day? Is there anything speaking in favor of this? I believe this is being done in some countries.

CLARKSON: Kehoe et al., 1943, studied a variety of compounds added to food, including ascorbic acid. Ingestion of varying amounts of milk and diets containing high and low calcium and phosphate were also studied. They were unable to detect any direct effects of these compounds or of the different diets. In the German lead study I referred to (Miler, Sattler and Menden, 1970) the protein concentration in the food was varied and differences in absorption rate were found. It is difficult to generalize because there are tremendous differences among different types of metals.

GOYER: From experimental studies on animals, similar to Kehoe's human studies, we were not able to show that increase in calcium above recommended daily intake did anything in terms of decreasing lead absorption. On the other hand we did learn that, if you reduce calcium in the diet to less than recommended amounts, there is an enhancement of lead absorption or at least increased body stores (Six and Goyer, 1970). In answer to Dr. Yllner's question I would say that one should be certain that the daily calcium intake is adequate in workers but not necessarily expect any beneficial effect from excessive intake.

CLARKSON: Figure 8 is taken from a study by Clarkson, Small and Nor-

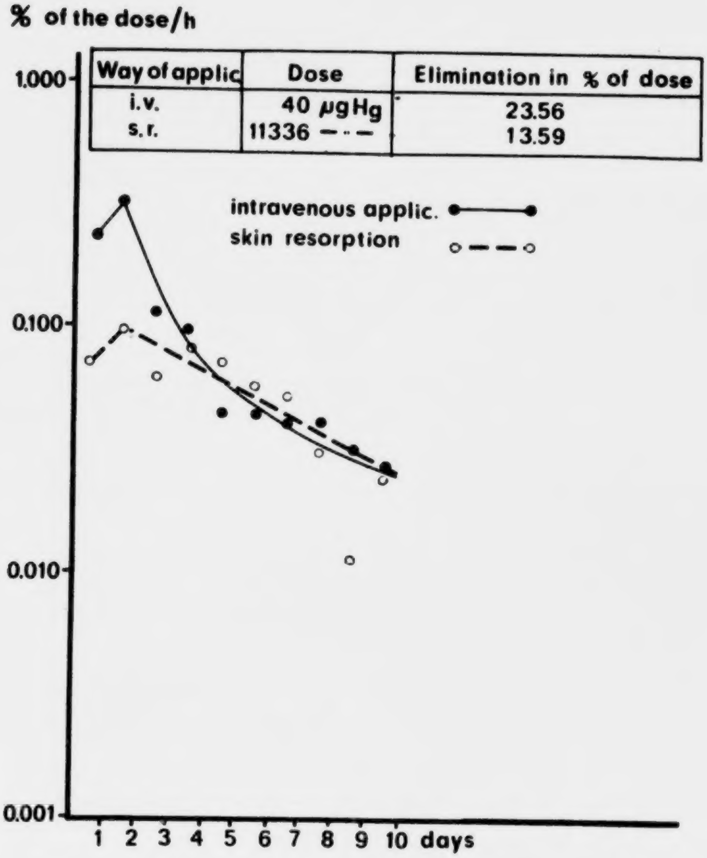


Figure 9. The Elimination of Mercury via Urine after Intravenous and Skin Application.

seth, 1971, and indicates three types of polystyrene resin added to food to test if the gastro-intestinal absorption of mercury could be reduced. The resins tested contained the following active groupings: 1) a quaternary nitrogen group, 2) carboxylate groups and 3) SH groups. The resin containing the thiol groups markedly inhibited the absorption of methyl mercury compounds. It also increased the fecal excretion by methyl mercury by trapping the metal secreted in the bile and thus preventing reabsorption.

Table 4. Elimination of Metals by Various Ways of Absorption. The Ratio Urine/Feces

Substance	Application	Dose in mg	Ratio urine/feces
Arsenic	Intravenous	0.200	8:1
	Skin	0.203	1:1
Beryllium	Intravenous	0.0043	3:2
	Skin	0.568	1:9
Chromium	Intravenous	0.050	1:1
	Skin	0.125	3:7
Mercury	Intravenous	0.040	4:3
	Skin	11.366	2:5
Selenium	Intravenous	0.116	4:1
	Skin	0.069	2:1

Synthetic resins may prove useful in reducing the gastro-intestinal absorption of other metals and may increase fecal excretion of those metals undergoing enterohepatic recirculation.

DUTKIEWICZ: I think that more attention should be placed on skin absorption as a cause of metal poisoning. There are several examples from laboratory investigations as well as from industrial conditions where skin absorption of metals is very essential in evaluating exposure. Animal experiments show that the rate of metal absorption via the skin could be quite important. Our data show that the rate could reach 10 to 1000 micrograms per cm^2 per hour or more, depending on the nature of the metal and on the concentration. Data in humans show similar values. I mean that in real life the cutaneous absorption could be equal or higher than that of the respiratory tract. For substances with a significant skin absorption, evaluating exposure based on concentration of the substances in air only is insufficient. In this case toxicological exposure tests should be elaborated, based on for instance the excretion of metals in urine, which could show the exposure independently of the route of absorption. Unfortunately there are great differences in the kinetics of elimination and in the ratio urine/feces excretion at various ways of application. Figure 9 indicates the varied character of excretion of mercury in urine after intravenous skin resorption. In Table 4 the ratio urine/feces excretion is demonstrated at various ways of application: intravenous and percutaneous, and there are great differences in the ratio. For arsenic, e.g. the ratio is one to one after skin absorption and after intravenous and intratracheal application 8 to 1. If urine analysis is used for evaluating exposure due attention must be paid to route of absorption.

NORSETH: I also have tested the effect of some macromolecules on absorption of methyl mercury from the gastro-intestinal tract. These are a thiolated sephadex (Eldjarn and Jellum, 1963) and thiolated dextran (Jellum, Aaseth and Eldjarn, 1971) which affect absorption, but which unfortunately are broken down to a great extent in the G.I. tract.

We have heard that heavy metals are probably bound to proteins in food. We have also heard that mercury molecules of certain kinds, that are probably not broken down and not reabsorbed, inhibit absorption. I would like to force the panel to answer how metals are absorbed, as free metals, as peptide bound metals or as amino acid bound metals?

CLARKSON: We do not know the mechanisms of absorption and we cannot therefore make any predictions. There are certain metals probably like the essential trace elements which have special mechanisms. In the case of iron there are special mechanisms. As far as lead, mercury and cadmium are concerned, the mechanisms are not known.

NORSETH: If we go back to the methyl mercury example again, this is interesting because there are at least four different forms of methyl mercury bindings in the intestinal tract. Methyl mercury in food is bound to proteins and is reabsorbed to 100 percent. Methyl mercury in bile is probably excreted as some kind of peptide and reabsorbed very efficiently. Some methyl mercury in bile is protein-bound and not reabsorbed that efficiently, but to a certain extent. I do not think that methyl mercury bound to proteins which are released in the intestinal tract by cell shed is reabsorbed at all.

CLARKSON: The explanation usually given for the efficient gastro-intestinal absorption of methyl mercury is that methyl mercury chloride is lipid soluble. This explanation is at least plausible in the case of absorption from the gastric contents where the concentration of hydrochloric acid may be high. However, methyl mercury in plasma, bile and other biological fluids is probably in the form of a complex with sulfhydryl containing proteins, peptides or amino acids. Methyl mercury in bile has been shown to be complexed to protein and to small organic molecules (Norseth and Clarkson, 1971, Norseth, 1971, in press). Such complexes are water soluble. The high chemical affinity of the mercury cation for the sulfhydryl group suggests that mercury chloride will be present in very small concentrations in tissues and biological fluids other than in the gastric contents. Thus, it is possible that special transport mechanisms, related to specific complexes of mercury, may be important in the transport of mercury across the intestinal, bloodbrain and placental barriers. Levels of mercury in brain and fetal tissue are lower after doses of phenyl mercury compounds than after doses of methyl mercury salts. The difference cannot be explained by differences in lipid solubility since both methyl mercuric chloride and phenyl mercuric chloride are soluble in lipids.

NELSON: I would like to make some points here in relationship to the question of active versus passive absorption in the two contrasting systems, the lung and the G.I. tract. I would expect that active absorption against a concentration gradient may be obtained fairly frequently in G.I. tract absorption. On the other hand I tend to relegate to a very secondary position the role of active transport in the respiratory tract.

WAHLBERG: I have two questions to answer. The first is about the mechanism of skin absorption, which we believe is passive diffusion in rodents and in humans. But an active absorption has been described through frog skin for sodium.

The second question was from Dr. Dutkiewicz. The figures I presented here concerned skin that was normal the seconds before I applied the metals. We think that the metals precipitate proteins in different ways and create new barriers and that is why the absorption is not so much increased as could be expected. Most workers in industry have small wounds. If the skin has been damaged by water, solvents, detergents and so on, we cannot speak of normal skin. This might explain the higher absorption that was described in the cases of poisoning. I think that measurements in urine are very rough measurements of skin absorption. We can see from the data presented by Dr. Dutkiewicz that the amounts applied on the skin were very much higher than those given intravenously, which explains the difference that was observed.

DUTKIEWICZ: The doses by intravenous application and by skin absorption of arsenic were equal and in spite of this fact, the differences in the ratio urine/feces were very important. According to the method of measuring skin absorption that Dr. Wahlberg uses, there is a disappearance of activity in the solution during for instance one hour. I think that there could be changes in the geometry of the measurement and there is a risk that Dr. Wahlberg's results may be wrong. I use another method in which the rats' tails are immersed in the solution for one hour whereupon the animals are put in metabolitic cages where urine and feces samples are collected every day for instance for 10 days. The absorbed dose is then calculated on the basis of excretion and on the content of elements in organs and tissues. In my opinion this method is better because the absorbed dose is measured here directly.

CHAIRMAN: With the exception of Dr. Wahlberg's presentation, so far we have discussed only mercury, cadmium and lead. That goes for the inhalation and intestinal absorption. Can I conclude that we know nothing about other metals or do you have some information you could give us?

NELSON: We, for example, have examined cobalt uptake in inhalation toxicity studies (Palmes et al., 1959). Uptake of cobalt fitted to a good approximation what could be anticipated from physical characteristics of the inhaled particles. In the few studies that have been done, I think that the model has been generally supported.

CHAIRMAN: Which model? Do you have an absorption model?

NELSON: The absorption model I am talking about is that most of the material reaching the alveolar part of the respiratory tract is "absorbed" according to the definition I gave earlier, while the amount of material reaching the alveoli can be estimated from particle deposition theory.

CHAIRMAN: I think that it would be a very useful conclusion if it really is true that, independently of the nature of the substance, if it reaches the alveoli, then it is absorbed to 100 percent.

NELSON: Although not necessarily 100 percent, it is generally high and in many ways equivalent to intraperitoneal or intravenous injection. Mobilization from that point is going to be dependent on the physical and chemical properties of the particles. Some materials, for example beryllium, have a high degree of tissue affinity and will be localized in the lung. Others will be less localized and will become systemically available. If one starts with the assumption that the material which reaches the alveolar part of the lung is absorbed in the sense I have defined, then one must go from this point and describe, in appropriately simple or complex ways, (depending on the material) what happens as it leaves the lung parenchyma. If it is very soluble, it may be immediately distributed and accessible to all tissues. If it is very insoluble, it will be localized. If it is reactive with local tissue, it may be sequestered, as in the case of beryllium. Materials that are relatively insoluble but not intensely bound may serve as depots for long-term discharge to other parts of the body.

CHAIRMAN: This means that certain substances may have biological half-lives in the lung for years. Others would have half-lives of weeks or months?

NELSON: Yes, this is dependent not on the mechanical properties, but on the chemical and biochemical behavior of inhaled dust. Some particles may end up in the lymph nodes where they may stay for a lifetime.

BERLIN: I would like to point to the fact that a small fraction of inhaled particles will be deposited in the upper respiratory tract, i.e. the nasal and the tracheal part. Very little is known about to what extent an absorption takes place for different particles and gases in the mucosa of the upper respiratory tract.

CHAIRMAN: Dr. Clarkson, would you be kind to comment on if you have any data on G.I. absorption for metals other than lead, cadmium and different mercury compounds?

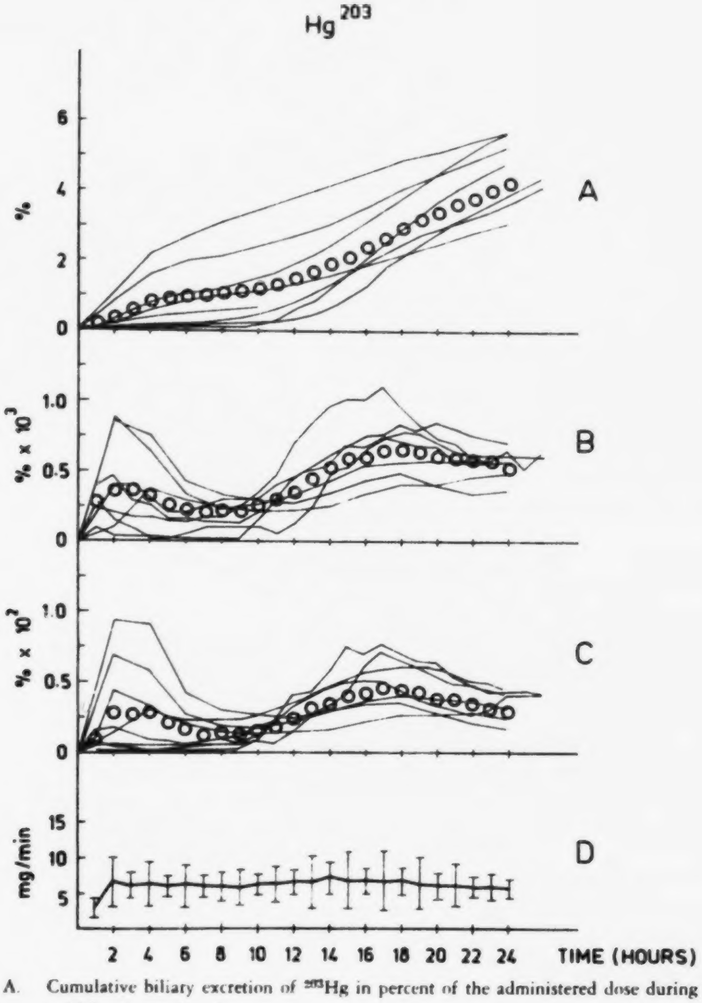
CLARKSON: There is a large number of papers concerning the absorption of physiologically important metals such as sodium, potassium and calcium. Iron absorption is carefully controlled by complex mechanisms acting in the mucosal epithelial cells. The exfoliation of epithelial cells plays an important role in the control of iron absorption (Crosby, 1968). In the normal individual, iron is absorbed from food into the mucosal cell. A substantial fraction is retained in the mucosal cells and re-excreted when the mucosal cells are lost by the exfoliation. A substantial fraction of the fecal excretion of mercury in animals dosed with methyl mercury salts originated from exfoliation of cells from the small intestine (Norseth and Clarkson, 1971). Complex interactions and interdependencies are known to occur among iron, copper and cobalt with regard to intestinal absorption. I understand that small molecular weight proteins similar to metallothionein are present in the intestinal cells, but perhaps Dr. Piscator would comment on this?

PISCATOR: We really have two problems here because, when talking about the lung, any essential or non-essential metal can be absorbed. For example, exposure via the air to manganese can be a serious health hazard. However, when manganese is in food we have a homeostatic mechanism which quite efficiently regulates the uptake. A worker exposed to copper will absorb the copper which is excreted via the bile, for instance. Under ordinary circumstances there is a reabsorption. In workers exposed to copper via inhalation the reabsorption of copper from the intestines will decrease, because of a homeostatic mechanism. In the intestinal walls of several species specific carriers have been found which are proteins of low molecular weight, very similar to metallothionein. These low molecular weight proteins, I think especially copper binding proteins, maybe also act as carriers for zinc and cadmium. We do not know that yet, but it is quite conceivable. From the intestines we know that iron has specific carriers. I do not think we know which carriers we have in the lungs.

CLARKSON: Miss Barbara Davis, a graduate student in our department, has been studying the homeostatic control of iron absorption. The control mechanism is effective in concentrations of iron normally encountered in food. However, when the oral dose is sharply increased, the homeostatic mechanism breaks down thus allowing a large increase in iron absorption. Fatalities in children who have taken overdoses of iron tablets are probably the result of the loss of homeostatic control. Thus we cannot expect that the normal physiological control mechanism will be life-saving when toxic doses of metals are ingested.

CIKRT: I have a short comment with regard to the excretion of metals via bile and their reabsorption in the intestinal tract. In our laboratory the biliary excretion of ^{52}Mn, ^{64}Cu, ^{203}Hg and ^{210}Pb after intravenous administration of $^{52}MnCl_2$, $^{64}CuCl_2$, $^{203}HgCl_2$, and $^{210}Pb/NO_3/_2$ was studied in rats. Cumulative biliary excretion 24 hours after administration reached in case of ^{64}Cu 31 percent, of ^{52}Mn 28 percent, of ^{203}Hg 4 percent and of ^{210}Pb 7 percent (each in percent of the administered dose). The maximum rate of excretion in the bile was reached in the individual metals during different periods after administration. In manganese the excretion was quickest with a maximum rate of excretion about 10 minutes after administration. ^{203}Hg differed significantly by the character of the excretion curve from three other metals (see Figure 10). On the B curve (percentage of excreted ^{203}Hg per mg of bile) you can see two peaks. The first peak is about 2—3 hours after administration and the second peak 17—20 hours after administration.

The bile was separated using the gel electrophoresis and we found that metals excreted via bile in high amounts (Cu, Mn) were located in the front of the electrophoreogram where the bile pigments and the proteins of low molecular weight are found, whereas Pb and Hg were located in the start of the electrotrophoreogram where the high molecular weight proteins are found. We obtained very similar results at separation of bile on Sephadex G-100. We also studied the reabsorption of biliary excreted manganese, copper and mercury and we found the following results: ^{52}Mn is reabsorbed to about 10 percent, ^{64}Cu to about 5 percent and ^{203}Hg to about 1 percent only.



A. Cumulative biliary excretion of ^{203}Hg in percent of the administered dose during a 24 hour experiment.

B. Percentage of excreted ^{203}Hg per mg of bile (Values × 10^3).

C. Excretion of ^{203}Hg in percentage of the administered dose per minute (Values × 10^2).

D. Bile flow in mg per minute. Solid lines—results in the individual experimental animals. Open circles—mean values of excretion.

Figure 10. Biliary Excretion of Mercury after Intravenous Administration of $HgCl_2$.

TSUCHIYA: I will refer to the absorption through the intestinal tract. In my experience, some workers exposed to lead sulphide did not show any clinical symptoms although the environmental lead concentration was rather high, exceeding 0.5 mg/m^3. Therefore, I think that not only the type of metal, but also the type of compounds are very important in absorption. For cadmium oxide fumes we have lower TLV-values than for cadmium dusts.

MALCOLM: I would like also to comment on this as not only the type of compound, but also the particle size are essential. In industry it is well recognized that cadmium fumes and lead fumes are absorbed much more readily than the ordinary dust. I think perhaps the TLV should be related to some extent to the profile of the particle size. On the other hand in practical work we find that lead or cadmium dust in the theoretical absorption range is absorbed only to about 20 percent. When you have it in fume form, in a 100 percent theoretical absorption range, the absorption is just about twice the level for a given concentration in the atmosphere. There is something missing here. I do think it is important to get more information on this, to be able to define TLV's in the particle size profiles. So far as lead sulphide is concerned, I have never been quite sure whether this is because of the nature of the compound or because very frequently the lead sulphide is in very large particle sizes and therefore not absorbed in the alveoli.

CHAIRMAN: 1) The next topic for our discussion is excretion and biological half-lives.

BERLIN: There are three excretion routes which should be considered the main routes, i.e. the alimentary tract, the urogenital route and the skin. In the alimentary tract there is a number of possible routes: salivary glands, stomach mucosa, small intestines, the liver, pancreas, the bile and the colonic mucosa. Although these routes are all possible, they may not be important in all instances. The excretion from the salivary glands, for example, is not very often significant but could be used for diagnostic purposes. Mercury is excreted that way and it may be used for evaluating the blood concentration. For such metals as cadmium, for example, which are retained for very long periods of time, this route could also be of importance. It is commonly observed in autoradiograms on distribution after injection of metals, and even other substances, that there is an excretion via the stomach mucosa. In Figure 11 you see the accumulation in the epithelium after intravenous injection of ^{109}Cd to a mouse and you

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1) At this point in the discussion, Drs. Clarkson and Nelson had to leave to catch their flights out of Slanchev Briag.

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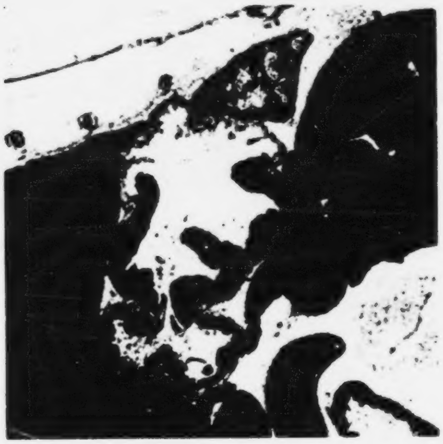

Figure 11. Accumulation of ^{109}*Cd in Stomach Mucosa after Intravenous Injection of* ^{109}*CdCl*$_2$ *to a Mouse (from Berlin and Ullberg, 1963).*

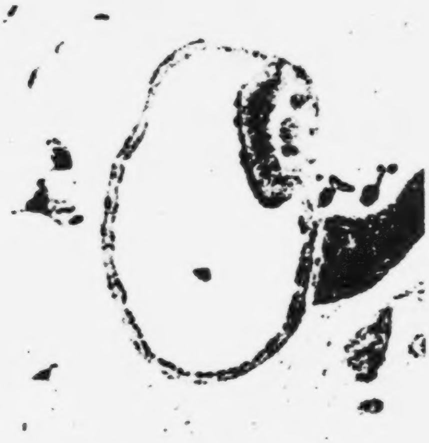

Figure 12. Accumulation of ^{109}*Cd in Colonic Mucosa after Intravenous Injection of* ^{109}*CdCl*$_2$ *to a Mouse (from Berlin and Ullberg, 1963).*

Table 5. Turnover Time of Intestinal Epithelial Cells (from Creamer, 1967).

Site	Species	Method	Turnover time (days)	References
Duodenum	Mouse	Labelling	2	Creamer, Shorter & Bamforth, 1961
	Rat	Mitotic counting	1.6	Leblond & Stevens, 1948
	Cat	Mitotic counting	2.3	McMinn, 1954
	Man	Mitotic counting	2	Bertalanffy & Nagy, 1961
	Man	Labelling	5—6	MacDonald, Trier & Everett, 1964
Jejunum	Mouse	Labelling	3	Leblond & Messier, 1958
	Mouse	Labelling	2	Creamer et al., 1961
	Rat	Mitotic counting	1.3	Bertalanffy, 1960
	Man	Labelling	5	Shorter et al., 1964.
Ileum	Mouse	Labelling	3	Leblond & Messier, 1958
	Mouse	Labelling	2	Quastler & Sherman, 1959
	Mouse	Labelling	1	Creamer et al., 1961
	Rat	Mitotic counting	1.4	Leblond & Stevens, 1948
	Cat	Mitotic counting	2.8	McMinn, 1954
	Man	Labelling	3	Lipkin, Sherlock & Bell, 1963

see a halo of an isotope just outside, indicating excretion. Similar results for mercury compounds have been observed.

The colon is a route which is overlooked to a large extent probably due to our limited possibilities, so far, to study this. It has been shown by several workers that mercury, for example, is excreted via the colonic mucosa. Cadmium is also excreted this way as you can see in Figure 12. It is rapidly accumulated in the mucous membrane and the free isotope can be seen in the lumen on the top of the epithelium. Of course, since these observations are made mainly shortly after the intravenous injection, the form of the metal in blood may be of importance. Later it may be in a form which is not excreted in the colon.

Another important route is the shedding of epithelium in the small intestines, where there is a tremendous turnover of cells. Table 5 shows the turnover time in different animals and in man as brought together by Creamer, 1967. The daily turnover time in man is about three times. As many metals are actually accumulating in the epithelium of the small intestines, the amount, which is liberated this way and probably excreted, cannot be unimportant, especially for metals like cadmium and others which may be retained over very long periods of time.

We have already discussed the excretion in bile, which also is an important route. The hepato-intestinal circulation which has been observed experimentally for several metals, such as lead, cadmium, mercury and manganese, may be a significant factor in considering our therapeutic possibilities. If you have such a circulation, it is logical to try to break that to get more excreted. This has been successfully done with methyl mercury experimentally as Dr. Clarkson has shown and I am sure it may be a possibility also for other metals.

In the skin, there is a number of important glands. The mammary glands are of course essential in pregnancy and especially for the new-borns. If the women are exposed to metals in industry, the excretion through the mammary glands could be an essential risk to the new-born. Since this is the only food intake, it would be a considerable daily intake. The other routes, like sweat glands and so on, are certainly of less importance, but can be used for diagnostic purposes. The epithelial shedding from the skin on the other hand should be of some importance for metals, such as cadmium where there also is this kind of accumulation in the skin.

Hair can often be a good indicator of body burden for metals and has been used as such successfully in epidemiological studies. In animal experiments a large part of the body burden can be found in the fur. In our studies on methyl mercury on squirrel monkeys for example the fur was found to contain more than 50 percent of the body burden after 2 months (Berlin, Nordberg and Hellberg, 1971, in press).

Kidneys are one of the most important organs for the excretion of metals. Maybe this is the best studied route. Epithelial shedding in the kidneys is another possible route for metal excretion, which has to be considered in cases where we have very low output. Perhaps semen could be of interest, especially from the point of view of genetic effects. The fetus is another important route of excretion in experimental animals and even in man this could be a way to lose a large part of the body burden of accumulated metals. In methyl mercury studies, for example in the hen, a considerable part of the body burden is lost via the egg.

I would like to turn to the question of biological half-lives and would like to stimulate a discussion here by saying that I do not personally believe that the biological half-life is really a useful concept in terms of evaluating risks. Only in very few exceptions where the excretion is so slow that any other turnover rate in the body is rapid in comparison to excretion, can we use this concept practically. In other cases I feel it is better to discuss the turnover in various organs, especially of course the turnover in critical organs. Since the effect is a matter of the dose at the site of action, it is therefore much better to discuss the turnover in the slowest organ. Which organs would show a slow turnover generally? The testicles have several times been shown to have a very slow turnover for cadmium, but not only for cadmium, but the same is true for mercury and polonium. Polonium is an alpha-emitter which is distributed in the testicles. We get a lot of polonium by cigarettes, for example. This could be of much importance from the point of view of genetical risks.

The brain has some parts with a very slow physiological turnover and it shows a slow turnover even for some of the metals deposited in the brain. This has been found for mercury and lots of facts indicate that there are other metals that have similarly slow turnovers, like manganese for example. Of course, the deposition in bone is related to the turnover of the bone tissue which again varies according to different physiological and pathological conditions. This was just to point out some of the important questions. I am sure there is a lot here that can be discussed.

TSUCHIYA: I will add a few comments on the relationship between absorption and biological half-life of cadmium. As you know there is a great disagreement with regard to biological half-lives of metals. For example, for cadmium, some animal experiments have shown values of only around 50 to 100 days. Of course, absorption and excretion may differ in different animal species and result in a great discrepancy in biological half-life. Nor have we reached an agreement in human observations. Kitamura (personal communication) made an observation on autopsy materials (on humans) and estimated the biological half-life of cadmium as 4 to 5 years. He also made an experiment on a human subject, and observed 5.3 percent absorption of cadmium from ingested water when starved, and 1.5 percent of absorption through the gastro-intestinal tract when rice was added with $CdNO_3$. As I have already presented in another session, we derived the biological half-life for cadmium as 16 years, based on the information about the total body burden, daily intake, and absorption via the G.I. tract as 3 μg, via respiratory tract as 1 μg.

When calculating the total body burden or biological half-life, it is most important to evaluate the methodology, including absorption rate. Usually in deriving the biological half-life we use short-term experiments over a period of some days, at most one year or so. For cadmium or lead however, we ingest very low amounts of cadmium daily over a period of many years, 50—80 years. Long-term ingestion with a low dose of cadmium may be quite different from short-term experimentation. Therefore I want to point out again that we have to appraise very carefully the methodology of how to calculate or derive a biological half-life of metals. We should collect more information in the future about the absorption and excretion which may vary according to the dose or concentration and the length of exposure as well as to host factors.

Dr. Berlin mentioned that the biological half-life may not be very important, but I do not agree, because from the point of view of total body burden we have to know the biological half-life in order to set adequate standards for metals in food and air in the future. I agree with Dr. Berlin on the point that there is a target organ. For example, lead is mostly accumulated in the bones and the biological half-life in bones and the total body burden might be different. Nevertheless I still think it is very important to know the biological half-life of many toxic substances.

CHAIRMAN: I do not think there is any disagreement between Dr. Berlin and Dr. Tsuchiya concerning a very long biological half-life. What Dr. Berlin mentioned was that he did not think that the measurements of the biological half-life were useful for those elements for which the turnover within the body is relatively slow in relation to the excretion. I am also in complete agreement with Dr. Berlin that the biological half-life in critical organs is of great importance. The question is how to measure the biological half-life in such organs. One could always do it in animal experiments, of course, but how do we approach that for humans, since we know that one cannot just extrapolate from animal experiments to humans?

BERLIN: If you know what organ you want to study, I think, there are several types of methods which could be applied. Some organs are reachable from the point of view of blood supply. One can measure output and input. Generally biopsies are not very realistic to use clinically, but for experimental studies on animals one can certainly use them.

One interesting organ is bone tissue. We have not exhausted our possibilities to find methods to determine different kinds of metals in bone. One method is to use X-rays with specific absorption characteristics for different metals. One of the most difficult organs to study is the brain. We do not really know the relationship between metal concentration for example in cerebro-spinal fluid and in brain tissue. The probable reason for that is that the amount in the cerebro-spinal fluid generally is very low and our analytical possibilities have been very limited. Rapid improvements have brought modern methods by which very low concentrations can be analyzed. As the cerebro-spinal fluid is accessible for measurements, we have some possibilities.

TSUCHIYA: But Dr. Berlin, if there is a close relationship between the concentration of methyl mercury in the brain and the total body burden of methyl mercury, you may know the biological half-life in a target organ by estimating the total body burden of mercury.

BERLIN: I guess methyl mercury is one of the metals for which we believe there is a relationship between body burden and the brain concentration. However, recent experimental evidences show that the transport and distribution of methyl mercury in brain are very slow processes. So far the crude clinical and experimental data still support the idea which is a good working hypothesis, that there is a correlation between brain concentration and body burden. It still remains to be proven however.

CHAIRMAN: If you take, e.g. inorganic mercury, what is the biological half-life in the brain? Would it be a couple of months, a couple of years or perhaps 20 years?

BERLIN: I would like to point out that the brain is not really one organ. It cannot really be considered as one type of cells or one entity metabolically. It covers a number of functions with different biochemical organizations, having been put together in a mass. We have to consider the brain as different kinds of centers, different metabolic activities which certainly are not morphologically organized from the point of view that you have one center here and one there. Since they are all mixed cells, we have to find a way to classify different kinds of biochemical systems in the brain. What system is affected and where do we have the metal? Some years ago in our laboratories we had some interesting results in guinea pigs showing tremendous accumulation in a few types of neurons in the brain stem as compared to the rest of the brain. It is very important to know what the half-life is in these neurons compared to the rest of the brain. I believe that one could easily have rather inert mercury deposited somewhere in the brain with no effect whatsoever. On the other hand the concentration in some areas may be very important where you may have another kind of half-life. We cannot really simplify it in saying that the brain is one entity with one common half-life.

CHAIRMAN: I appreciate that, but could you give any figures for any part of the brain or of total brain? Make a guess between half a year, two years or 30 years or something like that.

BERLIN: No, we do not know that much at the present, but we can say that we have some clinical cases which actually suggest half-lives, exceeding years. The question remains open.

TSUCHIYA: In my experience I have seen many cases of inorganic mercury poisoning, most of them, I think, reversible in clinical symptoms, i.e. the clinical symptoms will sooner or later disappear after removal from exposure. If you remove a worker with mercury poisoning for 4—5 months, his clinical symptoms such as tremor etc, may mostly disappear. At an earlier session I showed a figure in which I estimated the biological half-life of inorganic mercury. The half-life for total body burden would be around one month. But I do not know of course the biological half-life in the brain. This remains to be solved. But as long as organic changes in the brain do not develop, the decrease of excretion of mercury parallel the improvement of clinical symptoms.

NORSETH: It is unrealistic to talk about methods to determine the critical organ body burden in humans. I think that the last part of this discussion shows very clearly that we have not only to determine the critical organ burden, but also the critical cell burden, which is an impossible task. Therefore, let us look at it from a practical point of view. I would suggest that we do some animal experiments with doses, organ distribution, eventually site of action, distribution, if we can, and excretion, whereafter we go to humans to try to determine dose and excretion. Based on the kinetic studies in animal experiments and what we know about mechanisms, we make some kind of model for organ content in humans at different times. Then we take someone accidentally poisoned and study the organs to see if they fit the curves. This has been done for methyl mercury and the results combined with a few human experiments are the basis for our toxicological considerations related to this compound.

MALCOLM: From a clinical point of view there are certain practical difficulties with regard to methyl mercury, but also to lead. A lead paralyses can last a very long time. If it is due to the chelation of lead in the neuromuscular junction, there is some evidence that even quite long lasting paralyses has been improved by chelation. I am never quite convinced by this work, or have we not an accumulation of lead but an actually pathological damage to the cells which does not recover? I think we must differentiate between these two effects, either the presence of metal or pathology and brain damage. With methyl mercury you may get a permanent damage to brain cells which will not recover, even after the metal has disappeared.

GOYER: In the nervous system I am not sure that we really know which cells are involved in the partitioning of lead. In our laboratory Dr. Krigman and his group are working with suckling rats given a large amount of lead. He has found that there is an increased number of astrocytes and microglial cells. He has been able to give enough lead to form an intranuclear inclusion body in astrocytes at the periphery of the brain where it is in contact with cerebro-spinal fluid, suggesting that these are probably the cells that take up the largest burden of lead. But as far as clinical symptomatology and long-term effect are concerned, it is the neuron that is important. Studies on children have shown that even of those children that do recover from clinical encephalopathies a large percentage suffers to some degree from permanent damage, in some instances minor, but in others very severe (Perlstein and Attala, 1966).

With regard to Dr Malcolm's comment, I agree that there are probably two types of lead effects on the nervous system, one functional and reversible, the second resulting in permanent nerve cell damage. Experimental studies have shown that lead may interfere with transmission of impulses at preganglionic nerve endings by reducing output of acetylcholine output (Kostial and Vouk, 1957) so that this is a reversible type of lead effect. On the other hand, Schlaepfer, 1969, has suggested from his experimental studies that demyelination and Wallerian degeneration of peripheral nerves in lead nephropathy may result from a lead effect on the Schwann cell, the supporting cell of peripheral nerves.

DUTKIEWICZ: In the excretion of metals the kinetics is essential because when we know the character of the excretion and its mathematical model, one can calculate the absorbed dose and period of time necessary to reach the steady state. By various ways of absorption the kinetics of elimination changes according to the way of absorption. The problem is not that simple. Selenium elimination in urine after both intravenous and intratracheal application can be shown as a straight line, that is, it has a single phase elimination, whereas after skin application, the elimination is two-phased. It is similar for many other metals such as, mercury, chromium, arsenic, etc.

LEWIS: One phenomenon that has been touched on this morning, but not discussed in detail, is the proportional redistribution of trace metals within the body with time. Bonnell, Ross and King, 1960, give tissue concentrations of cadmium in liver and kidneys obtained at necropsy from a small series of men who had died of chronic cadmium poisoning, together with years of exposure and interval between last exposure and death. The longer the survival of a patient following exposure the smaller the liver/kidney cadmium ratio observed, indicating that cadmium is removed more rapidly from the liver than from the kidneys. Similarly, the data I presented yesterday, relating cadmium accumulation to cigarette smoking, showed that the mean cadmium content of lungs derived from ex-smokers was similar to that of non-smokers. In contrast, when mean kidney values were considered ex-smokers identified themselves with smokers. It is therefore, extremely important to define the distribution dynamics within specific organs, when considering the kinetics of trace metal distribution and elimination particularly when possible associations with disease, social habits and occupation are being sought.

PISCATOR: In another session here we showed how the kidney was accumulating cadmium for months with a simultaneous slow decrease in the liver after just one single injection. Dr Berlin mentioned earlier with regard to excretion routes that it is quite conceivable that after injection, cadmium will at first be in a low molecular form and excreted in some part of the intestines. In a later phase the main part of cadmium is stored in the liver. We have found that in mice after several months the concentration is higher in the pancreas than in the liver, meaning that accumulation and excretion are taking place also in the pancreas.

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SECTION III

ACCUMULATION OF TOXIC METALS WITH SPECIAL REFERENCE TO THEIR ABSORPTION, EXCRETION, AND BIOLOGICAL HALF-TIMES

Prepared by the

Task Group on Metal Accumulation

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SECTION III
ACCUMULATION OF TOXIC METALS WITH SPECIAL REFERENCE TO THEIR ABSORPTION, EXCRETION, AND BIOLOGICAL HALF-TIMES

III.1. BACKGROUND

At an international symposium on maximum allowable concentrations of mercury compounds in Stockholm, 1968 (MAC 1969), it was considered advantageous to form a group experienced in metal toxicology. At the meeting of the Permanent Commission and International Association on Occupational Health in Tokyo, 1969, it was decided to form a subcommittee on the toxicology of metals under this organization. This subcommittee (chairman, L. Friberg, Stockholm) organized a panel discussion dealing with absorption and excretion of toxic metals (Dukes & Friberg 1971) at the congress of the Permanent Commission and International Association on Occupational Health in Slunchev Bryag, Bulgaria, September, 1971.

At the meeting in Bulgaria, certain principles concerning absorption of metals via the pulmonary, gastrointestinal and cutaneous routes were discussed, as well as certain characteristics concerning the excretion of metals. It appeared to the subcommittee from these discussions that it would be worthwhile to examine the possibility of establishing models for some forms of absorption which would have general validity for several metals. As a result of these considerations, a working group convened in Buenos Aires, September, 1972, prior to the XVIIth International Congress on Occupational Health.

All participants had prepared working papers dealing with specific topics to be discussed, and these working papers had been circulated among the participants in advance of the meeting.

The meeting, which took place at the Ministry of Social Welfare, Public Health Area, Buenos Aires, Argentina, was officially opened by Dr. Adolfo Antoni, President of the Organizing Committee of the XVIIth International Congress on Occupational Health together with Dr. Lars Friberg, Chairman of the Subcommittee on the Toxicology of Metals under the Permanent Commission and International Association on Occupational Health. Dr. Lars Friberg of Sweden was elected Chairman of the meeting, Dr. Ana Singerman of Argentina was elected Vice-Chairman and Dr. Gunnar Nordberg of Sweden was elected Rapporteur.

The discussion at the meeting was centered on a "skeleton report" prepared by Dr. Nordberg mainly from the working papers (section 10). Some sections in the present report emerged from a detailed scrutiny of sections in the "skeleton report", and other sections were introduced and written during the meeting. The complete report was read, discussed and approved by the "Task Group" as a whole, but the detailed preparatory work for each section was performed by four working groups given responsibility for the specific sections, i.e. "Fundamental aspects of metal toxicity; gastrointestinal absorption and excretion" was dealt with by a group chaired by Dr. T. Norseth; "Absorption by inhalation" by a group chaired by Dr. R. Albert; "Transport, distribution, placental transfer and renal excretion" by a group chaired by Dr. J. Vostal; and "Accumulation and retention in critical organs and indices of exposure and retention" by a group chaired by Dr. M. Berlin.

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III.2. FUNDAMENTAL ASPECTS OF METAL TOXICITY

III.2.1. CONCEPTUAL CONSIDERATIONS

Certain metallic elements are necessary for the normal biological development and function of human beings and are known as "essential metals". Others may not be identifiable as serving a beneficial biological function and are known as "non-essential". However, the concept of essentiality is under constant review and an unequivocal classification of metals according to it cannot yet be made. All metals have the potential to cause adverse effects in human beings at certain levels of exposure and absorption. Mechanisms exist for regulating the body levels of some metals, mainly essential ones, but for certain unusual routes of exposure such protective mechanisms may be inadequate and for other metals they do not exist at all.

This report will be concerned mainly with mechanisms of absorption, distribution and excretion, with particular reference to biological half-times and accumulation. Primary consideration will be given to cadmium, lead, mercury and their compounds. Although the term "heavy metals" is frequently encountered in literature on metal toxicology, it will not be used in this report. Elements included by this term vary among authors, and the principles of absorption, excretion and accumulation dealt with in this report may apply also to elements not included in a particular definition of heavy metals.

III.2.2. BIOCHEMICAL CONSIDERATIONS

A toxic effect arises because certain biochemical reactions in the body become altered by the metal in question. Other mechanisms may operate but these will not be discussed.

Metals may produce structural changes in the cell membrane, affecting its permeability. When the metal reaches the inside of the cell, a number of enzymatic activities can become inhibited. Such interference of metals with enzyme systems may give rise to functional impairment. The enzymatic inhibition may involve an interaction at the active center of an enzyme, but the inhibition may also result from interactions between metals and ligands not directly involved in the active center. Changes in electrostatic charge may occur along with a shift in the ionization constant of the active center. Also conformational changes in a protein or the combination of the metal with a coenzyme may provide the basis for impairment of the enzymatic reaction.

Another way by which exposure to a potentially toxic metal may cause adverse effects is by changing the metabolism of other metals. Some tissues may be depleted of ligands or metals necessary for enzymatic function even if excessive amounts of the toxic metal are not stored in that tissue.

Metal binding occurs not only at sensitive sites in enzymatic systems of the cell, but also at sites in the cell where the metal is less active from a toxicological point of view. If there is a sufficient reserve of enzymes in a cell or additional enzymatic activity can be induced, inhibition of a portion of a particular enzyme within a cell will usually not impair cell function seriously. If an interference occurs with enzymes necessary for the build-up of ATP or other high energy nucleotides, some of the cell functions requiring little energy may continue as normal whereas functions requiring stored energy will be impaired. For several biochemical reactions in the body, important functions might continue by means of an alternative pathway. Usually, such pathways are less favorable and might produce an extra load on other systems in the cell.

III.2.3. CRITICAL ORGAN CONCEPT AND CRITICAL CONCENTRATIONS IN CELLS AND ORGANS

With the accumulation of metal, when undesirable functional changes, reversible or irreversible, occur in the cell, it can be said that the "critical concentration" has been reached. Any increase beyond the "critical concentration" is accompanied in most cases by increasing impairment of cellular function. The cellular concentration sufficient to cause cell death is the "lethal concentration" for that cell.

Analogously, the "critical organ concentration" is defined as the mean concentration in the organ at the time any of its cells reaches critical concentration. The critical organ concentration may be considerably higher or lower than the critical concentration for a particular cell.

The concept of a "critical organ" refers to that particular organ which first attains its critical concentration of metal. The organ of greatest accumulation or retention is not necessarily the critical organ.

Metabolic factors and sensitivity of organs show interindividual variability that is so great as to make one organ critical for one person and another organ critical for another. Epidemiological considerations pertaining to variations in the "sensitivity" of individuals to a particular metal at a certain level of exposure are therefore of interest.

It is not the intention in this report to discuss which specific concentrations of various metals are critical for various organs. However, in general, the information contained in Table 6 cites the organs which are critical following exposure to cadmium, lead and mercury.

Table 6

Organs critical in metal intoxication

Cadmium (Cd^{2+})	Lung Kidney Liver
Lead (Pb^{2+})	Hematopoietic tissue Central and peripheral nervous systems Kidney
Tetraethyl lead $(C_2H_5)_4Pb$	Central nervous system
Triethyl lead $(C_2H_5)_3Pb$	Central nervous system
Mercury vapor (Hg^0)	Central and peripheral nervous systems Kidney
Mercury (Hg^{2+})	Kidney
Methoxyethyl mercury (CH_3-O-$CH_2CH_2Hg^+$)	Kidney
Phenyl mercury ($G_6H_5Hg^+$)	Kidney
Methyl mercury (CH_3Hg^+)	Central nervous system

These considerations show that it is of importance to determine which organs and which groups of cells within them are most susceptible to damage when metals accumulate there. Subcellular partitioning of metals among various organelles and intracellular fluid may also be of considerable importance. It is now known for some metals that specific proteins or classes of proteins which have preferential affinity for the metal are present in cells. These proteins may bind a particular metal so that it is not available to interact with normal cellular functions or enzyme activity. Such protein binding might detoxify the metal. It becomes apparent that not only the cellular concentration of a particular metal, but also its intracellular location, form, and whether it is free to react in the cell, are critical. Other factors which may determine the efficacy of the protein-metal complexing reaction in any particular cell at a specific time are age of cell, functional demands, nutritional state, rate of exposure to metal, chemical form of metal, etc.

The proteins which bind metals to detoxify them may not be present in all cells of the body at all times. It has been shown that such proteins (e.g. metallothionein) may be inducible so that availability to bind with metals may vary. Similarly the fate of such metal-protein complexes may vary, that is, the rate at which they are catabolized or excreted. These considerations suggest, therefore, that critical concentrations of a metal might vary depending on the extent of formation of inactive chemical forms of metals within cells.

It is not within the scope of this report to consider the accumulation of metals in relation to mutagenesis or teratogenesis at subcellular, cellular or organ levels. Furthermore, this report will not deal with the effects of radiation emanating from metals.

III.3. ABSORPTION

Absorption is defined as entry into the body by passage of the metal or its compound across a membrane. The metal compounds may be retained at the local site of absorption for long periods of time or they may be transported elsewhere.

The two principal routes for absorption are inhalation and ingestion and these will be discussed in detail. Under certain circumstances, the skin is an important route by which metals enter the body. This type of absorption was dealt with to some extent at the previous meeting of the subcommittee on the toxicology of metals (Wahlberg, in Dukes & Friberg 1971), but will not be discussed in this report. Placental transfer is of importance for effects on the fetus. Knowledge of this form of absorption makes it possible to evaluate the contribution to the body burden at birth. Since the placental route of absorption has not been dealt with previously at the subcommittee's meetings, it was decided to include a short discussion in the present report.

III.3.1. ABSORPTION BY INHALATION

III.3.1.1. Introduction

Pulmonary absorption of metals is usually the most important route for their entry into the human body in industry. This absorption route is also of importance in exposure from the general environment, including exposure to tobacco smoke. Metals may be taken into the respiratory system in the form of particles, gases or vapors.

III.3.1.2. Absorption of metals in particle form

III.3.1.2.1. General principles

Absorption of metals and metal compounds in particle form is influenced by three processes in the lung, namely, deposition, mucociliary clearance and alveolar clearance. A model of these three processes is depicted in Fig. 13. After deposition in the nasopharyngeal, tracheobronchial or alveolar (pulmonary)

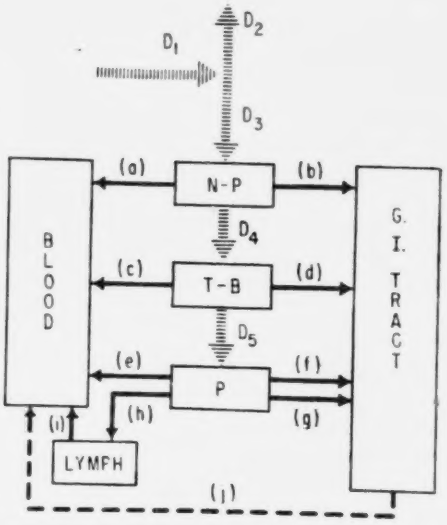

Figure 13. Respiratory tract clearance model (From Task Group on Lung Dynamics 1966).

N-P = Nasopharyngeal compartment; T-B = Tracheobronchial compartment; P = Alveolar (Pulmonary) compartment

D_1 = total dust inhaled.
D_2 = dust in the exhaled air.
D_3 - amount of dust deposited in the N-P.
D_4 = amount of dust deposited in the T-B.
D_5 - amount of dust deposited in the P.

a rapid uptake of material deposited in the N-P directly into the blood; b -- mucociliary clearance from N-P to G-I tract; c rapid uptake of material deposited in T-B directly into the blood; d - mucociliary clearance; e = direct absorption of dust from the alveolar compartment to blood; f inclusion of alveolarly deposited particles by macrophages and their transport by the mucociliary escalator to the G-I tract; g = same as f, but slower process; h = slow removal of particles by the lymph system; i - transport of dust cleared by (h) into the blood; j = G-I absorption of particles cleared to the G-I tract by processes h, d, f, and g.

compartments, the metal may be transported by mucociliary action to the gastrointestinal tract or absorbed into the blood. The fraction deposited in the non-alveolar compartments of the lung participates mainly in the first named type of translocation. The portion deposited in the alveolar compartment is more likely to be absorbed, but may take different routes.

III.3.1.2.1.1. Deposition of particles in pulmonary compartments. Deposition occurs by three physical mechanisms: inertial impaction, gravitational sedimentation and diffusion. Impaction and sedimentation depend on particle mass (size and density); diffusion is important only at particle diameters of less than a few tenths of a micrometer. Impaction is the dominant mechanism for unit-density particles larger than a few micrometers; it is also dependent on the velocity of the airstream entering the respiratory tract. Impaction occurs at sites where the airstream bends sharply and is the most important deposition mechanism in the nose, mouth, pharynx and upper bronchial tree. Gravitational sedimentation also depends on particle mass as well as the residence time of airborne particles in the lung. Sedimentation is an important mechanism in the smaller bronchi and alveoli for unit-density particles in the range of a few tenths to a few micrometers.

It is evident that deposition of inhaled metal particles (as is true for particles of all types) depends principally on their physical characteristics as well as on the patterns of respiration including the route of inhalation, whether nose or mouth, and the tidal volume and respiratory rate.

Deposition in the three compartments for particles with various mass median aerodynamic diameters has been estimated by the Task Group on Lung Dynamics, 1966, and is given in Fig. 14 for a tidal volume of 1450 ml, 15 respirations per minute, which represents moderate physical activity. The estimations in Fig. 14 were made on the basis of certain assumptions about the "standard" human lung and were only partly based on experimental data. The shaded areas in this figure indicate the calculated ranges of deposition that would result from variation of the physical characteristics of an aerosol in the lungs of the ICRP "standard" man. Observations show that in the particle size range 2-5 μm the average deposition pattern fits the ICRP model, but that individual variation is large, of the order of two to threefold (Lippmann et al. 1971). Individual differences in aerosol deposition have also been shown for animals (Holma 1967, Albert et al. 1968) and these individual differences appear to be stable (Albert et al. 1968, Tomenius 1973).

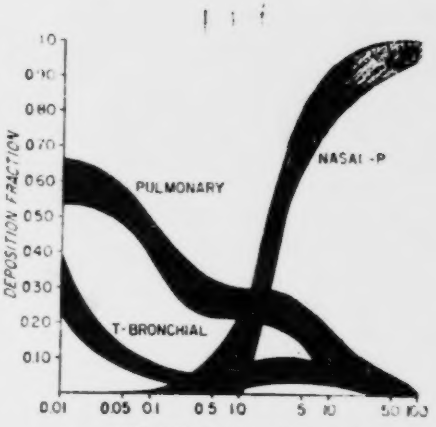

Figure 14. Deposition of particles in the lungs of "the Standard man" (from Task Group on Lung Dynamics 1966).

III.3.1.2.1.2. Mucociliary clearance. **Mucous flow** and ciliary activity in the tracheobronchial and nasopharyngeal systems constitute the process of particle transport designated as mucociliary clearance. The ultimate result is the translocation of material into the gastrointestinal tract or its expectoration. Because of the relative rapidity of mucociliary clearance, usually completed in less than 8 hours (Albert et al. 1971), the portion of the total deposition that falls within the tracheobronchial and nasal compartments is generally not absorbed into the body to any considerable extent, unless the aerosol particles consist of very easily soluble salts. Data to substantiate absorption of metal compounds by these routes are lacking.

Measurement of the amount of radioactivity in the lung at various times after the inhalation of an aerosol tagged with a radionuclide has been used for studies on "lung clearance". As a rule, curves obtained by such measurements may be simplified into at least two phases, an initial fast phase usually taking between one and 24 hours (usually less than 8 hours) and a second, slower phase. The first phase is considered to reflect "tracheobronchial clearance", a result of mucociliary activity. The second phase is considered dependent upon clearance from the alveolar compartment.

The first phase of lung clearance, "tracheobronchial clearance", is dependent upon particle size in that larger particles appear to be cleared more quickly than smaller ones due to their differing deposition and consequently different distances to travel up the tracheobronchial tree. Another factor that increases the difference is the higher speed of the mucous flow in the upper parts of the tracheobronchial tree compared to that in the lower regions.

Considerable differences among individuals in the tracheobronchial clearance of particles of the same size have been demonstrated in persons inhaling the aerosol under standardized conditions (Camner et al. 1971, Lippmann et al. 1971). These differences seem to be genetically dependent since monozygotic twins have clearance patterns very similar to each other, whereas dizygotic twins do not display more similar clearance rates than persons in general (Camner et al. 1972d). The tracheobronchial deposition and clearance studies show that certain persons have higher alveolar deposition and thus potentially higher absorption than others.

III.3.1.2.1.3. Alveolar clearance. Alveolar clearance consists principally of three different processes:

1. Transport of material from the alveolar compartment onto the mucociliary escalator and its subsequent translocation to the gastrointestinal tract or its expectoration.
2. Passage of the metal through one of the membranes into pulmonary tissues (where it will remain for a long time).
3. Passage of the metal through the pulmonary tissues into lymph and blood, and its translocation to other tissues by the systemic circulation.

Possible pathways for these processes include penetration through the junctions of the alveolar pneumocyte, interstitial space and the terminal lymphatics. Material which does not follow the interstitial space may be phagocytized by the interstitial macrophages, or may penetrate the endothelium of the blood capillary. It is not known which of these pathways is the most important. Phagocytosis by the alveolar macrophages is probably the most important mechanism for the transfer of particles to the mucociliary escalator (Morrow 1972).

It is not known for particles with low solubility, neither in human beings nor animals, how large a part of the alveolar clearance results from transfer of particles to the mucociliary escalator and how much is absorbed through the lung. Soluble metal compounds will be absorbed quickly, which will make them less available for mucociliary transport. However, it should be appreciated that with a long residence in the lung, even substances usually considered insoluble may undergo dissolution. Furthermore, solubility in lung fluids may be quite different than in water. For the part of the dust retained in the alveoli, a solubility model may be the most accurate means to evaluate the rate of absorption (Mercer 1967, Morrow 1970).

The Task Group on Lung Dynamics (1966)

categorized inorganic substances according to their predicted retention in the lungs. It is difficult to know the accuracy of this approach and to judge the reliability of the classification, since it was documented by experimental data to only a limited extent.

Where a substance is poorly absorbed from the gastrointestinal tract, the translocation of particles from the alveoli to the gut will minimize absorption. To illustrate this point, a hypothetical example is shown in Table 7, comparing the absorption for the case of no translocation but complete alveolar absorption with the case of 50% alveolar absorption, 50% translocation from the alveoli to the gut and 5% intestinal absorption. In both cases the entire tracheobronchial deposition is translocated to the gut and the alveolar and tracheobronchial deposition behave according to the ICRP model (Morrow 1970). With no translocation, the total absorption ranges from 9–50% with decreasing particle size. With 50% translocation there is substantially lower absorption, with a range of 7–27% at all particle sizes. This illustrates the importance of defining the extent of translocation from the alveoli particularly for metals which have a low intestinal absorption.

III.3.1.2.2. Specific experimental data on absorption of metals after aerosol exposure

For cadmium, lead and mercury, the metals to be treated specifically in this report, data on transpulmonary absorption are scarce. Friberg et al. (1971) reviewed observations on cadmium uptake in humans exposed to this metal and concluded that quantitative data were not obtainable. From various acute and chronic animal exposures, absorption amounting to 10–40% of inhaled cadmium could be estimated. As the gastrointestinal absorption of cadmium is relatively small, it was concluded that the major part of this absorption was transpulmonary.

For lead, a study by Hursh et al. (1969) provides data on the absorption in human beings of a ^{212}Pb-aerosol obtained by the decay of thoron to ^{212}Pb absorbed onto the natural aerosols of room air. The particle size of the inhaled aerosol was unknown, but the authors assumed a mass median diameter of 0.2 micrometers. Deposition of lead in the lung varied from 14 to 45% of the amount inhaled, and less than 8% was deposited on the tracheobronchial tree. The authors calculated a half-time of 6.5 hours for the absorption of lead from lung to blood. However, these data

Table 7

Calculation of total absorption into the body as a function of two different rates of alveolar absorption and different particle sizes for a specific deposition and clearance model. Gastrointestinal absorption presumed to be 5%

Particle size (MMAD)[a] µm	Alveolar deposition (%)	Tracheobronchial-nasopharyngeal deposition (%)	Total absorption (%) into body when alveolar absorption is	
			100%	50%
0.1	50	9	50.4	26.7
0.5	30	16	30.8	16.6
2.0	20	43	22.2	12.6
5.0	10	68	13.4	8.6
10.0	5	83	9.2	6.8

[a] mass median aerodynamic diameter.

are not necessarily applicable to ordinary industrial lead exposure due to the unusual physical and possibly chemical properties of the inhaled ^{212}Pb.

Specific data on transpulmonary absorption of inorganic and organic mercury compounds are lacking, although it is known from both animal experiments and human exposures that amounts of several of the compounds which are sufficient to cause poisoning can be absorbed.

III.3.1.3. Absorption of metals in gas and vapor form

The only general principle known for the deposition of gases or vapors in the respiratory tract is the dependence upon water solubility. Highly water soluble gases and vapors are dissolved in the mucous membranes of the nasopharyngeal and tracheobronchial systems and never reach the alveoli. For example, Strandberg (1964) showed that only a relatively small fraction was present in the inspired air at the tracheal level in rabbits exposed to sulfur dioxide. Those gases and vapors which are less soluble in water may not be dissolved in this way and therefore reach the alveoli. Depending on their ability to penetrate the alveolar structure, they are absorbed to varying degrees. Since the alveolar cells are covered by a phospholipid layer, lipid solubility of the gases and vapors will probably be of importance for their penetration of the alveolar membrane.

Most of the evidence available for metals concerns mercury vapor, probably the only case in which vapor exposure to a metal in elemental form is of any practical importance. Other metals occur in the form of vapors at very high temperatures. These vapors may cause certain occupational problems, but at the time they are inhaled the temperature is much lower and they have then become particles. It may well be that the particles are very small; nevertheless, this type of exposure falls within the above section on particulate matter. However, for various compounds of metals, especially some organometallic compounds, the vapor pressure at body temperature is high so as to make possible the inhalation of toxic concentrations of vapors.

Mercury vapor is nonpolar and easily penetrates the alveolar membrane into the blood (Magos 1967, Berlin et al. 1969a). Vapors behaving like mercury which are not dissolved in mucous membranes of the nasopharyngeal and tracheobronchial tracts, but are quickly transferred through the alveolar membrane, will be absorbed to an extent of about 80% of the inhaled amount. This percentage is a result of the quantitative relations between inspiratory volume and the physiological dead space of the lung (Nielsen-Kudsk 1965). Specific data for the transpulmonary absorption of organometallic compounds of lead and mercury are lacking, but it is generally believed that the absorption is high, i.e. about 80% of the inhaled amount.

III.3.1.4. Effects of age and pathological conditions

Information in this area is limited. No systematic data exist on the effects of age, particularly whether children differ significantly from adults with regard to deposition and clearance.

Nasal obstruction increases mouth breathing, which tends to increase the total pulmonary deposition of inhaled particles. Bronchial deposition in chronic bronchitics with airway obstruction is markedly increased probably due to the narrowing of the airways (Albert et al. 1973). This is probably also true for asthmatics. Such an effect would tend to decrease alveolar deposition.

Mucociliary clearance in cigarette smokers is slower than normal, which might facilitate the absorption of soluble aerosols deposited in the bronchi (Albert et al. 1969, Camner & Philipson 1972). Chronic obstructive lung disease (chronic bronchitis) also has been shown to decrease the rate of tracheobronchial clearance (Toigo et al. 1963, Camner et al. 1972a). Acute infectious bronchitis markedly slows bronchial mucociliary clearance (Camner et al. 1972b, c).

Little information exists on the influence of pathological conditions of the lung on alveolar clearance. There is inconclusive experimental evidence on the effects of emphysema on alveolar clearance (Ferin 1971). On the basis of dust burdens in autopsied miners' lungs, Davies (1963) has suggested that there is progressive impairment of the clearance of dust from the alveoli with increasingly severe pneumoconiosis.

III.3.2. ABSORPTION BY INGESTION

III.3.2.1. General aspects

A major source of metals entering the gastrointestinal tract is by the ingestion of food and beverages, but a substantial part of metals in particle form deposited in the lung may be transferred to the gastrointestinal tract by mucociliary clearance. A scheme illustrating the principal routes taken by metals introduced in the gastrointestinal tract is shown in Fig.15. The uptake of metals originating from the mucociliary clearance of the lung is governed by the same mechanisms as for the metals ingested, but the rates of absorption may sometimes differ. The transfer of metals from the intestinal lumen into the cells of the epithelial lining of the gastrointestinal tract (absorption) may not lead to further transport into the organism. The presence of metals as soluble salts or complexes is usually a prerequisite for absorption, but endocytosis of macromolecules or particles may be of some importance, as demonstrated in young animals (Sanders & Ashworth 1961).

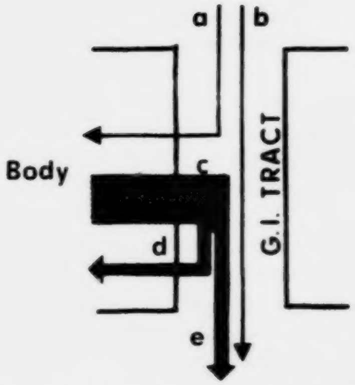

Figure 15. Principal routes for metals introduced into the gastrointestinal tract.

a = metal absorbed into the body.
b = metal introduced in g.i. tract but passing unabsorbed through the g.i. tract.
c = G-I excretion of metal.
d = metal G-I excreted and reabsorbed.
e = net gastrointestinal excretion.
b + e = fecal content of metal.

The rate of absorption of metals from the gastrointestinal tract is dependent upon their chemical form. Some organometallic compounds are absorbed to a greater extent than their corresponding inorganic compounds (Clarkson 1971). Anions are important for the degree of absorption only to the extent that they influence the solubility of metal compounds in the acid medium of the stomach (Magos 1972b, Pfitzer 1972). After transfer to the small intestine, metals are usually bound to various organic molecules and the anions become of minor importance.

Gastrointestinal secretions and digestive enzymes are important for converting the metal to a form available for absorption. Shifts in pH may change the valence form of the metal. It may also increase its solubility or ionization which can promote absorption after the formation of a complex more suitable for absorption. The absorption of metals varies depending upon location in the gastrointestinal tract, on functional variations, including differences in passage time, and on the patterns of intestinal microbiological flora.

Metal interaction may influence absorption processes as demonstrated by *in vitro* experiments with manganese, zinc, mercury and cadmium (Sahagian et al. 1966, 1967). Reduction of dietary calcium increases the absorption of lead and cadmium (Fleischman et al. 1968, Six & Goyer 1970, Larsson & Piscator 1971, Kobayashi et al. 1971, Piscator & Larsson 1972). The great demand for calcium in children, pregnant and lactating women will cause high absorption rates for calcium, which may increase the absorption of cadmium and lead. Similarly, lowering dietary iron increases lead absorption (Six & Goyer 1972). Difference in lead absorption between the newborn and adult may also reflect differences in gastrointestinal function such as capacity to absorb molecules of certain size, and quality and quantity of digestive enzymes and biliary excretion.

The protein content in food may influence metal absorption. Low protein in an otherwise isocaloric diet increased lead uptake (Milev et al. 1970).

III.3.2.2. Specific data

Data on gastrointestinal absorption (transfer of metals from the gastrointestinal tract to blood) have been listed for all metals by ICRP (ICRP publication 2, 1959). With regard to radiation protection, these values have served a good purpose. However, many entries were not based on experimental observations in human beings and sometimes only on limited animal data. For some of the metals which are of specific toxicological interest, including those metals chosen for particular focus here, the ICRP data are unreliable. They will not be discussed further in this report.

Cadmium. Studies on the uptake of inorganic cadmium compounds in animals (reviewed by Friberg et al. 1971) have shown retention after about 1 week of up to 3% of the ingested dose. Balance studies on "normal" intake of inorganic cadmium compounds via food in human beings theoretically give figures on whole-body retention of the metal, but to derive a precise figure is difficult for cadmium because of the low and variable intake of this element and the low gastrointestinal absorption.

Using ^{115m}Cd, Rahola et al. (1971) determined the retention of cadmium in five male volunteer subjects (ages 19–50) after administration of ^{115m}Cd mixed with calf kidney proteins. Whole-body counting as well as analysis of fecal and urine samples revealed that approximately 6% was retained after 10–15 days. It may well be that the gastrointestinal absorption as defined above is higher than 6% because some of the cadmium excreted within 10–15 days was absorbed into a body compartment with a very short half-time (a few days), as discussed below for mercury. The final interpretation of the data by Rahola et al. must await further data.

Lead. Gastrointestinal absorption of lead in humans, measured by determining the difference between lead ingested and lead eliminated in feces and urine, is between 5 and 15%. These data, although carefully derived, were only from a few normal adults and give no information about variation related to sex, age, and the large number of other factors known to influence metal absorption (see section 3.2.1) (Kehoe 1961). That such aspects should be taken into consideration is shown by an experiment made by Hursh & Suomela (1967). They measured lead absorption in three human volunteers exposed to ^{212}Pb and found a range of 1.3 to 16%.

Experimental studies have shown that absorption of lead is age dependent. Newborn rats have a higher absorption of lead when compared to adult rats (Kostial et al. 1971a); this may be related to the higher absorption of calcium in young rats. Greater retention of trace amounts of dietary ^{212}Pb occurred in 5- to 7-day-old rats fed cow's milk only than in rats fed cow's milk supplemented with calcium and phosphate (Kostial et al. 1971b).

Mercury. Inorganic mercuric $^{203}Hg^{2+}$ was administered orally either in the free or in the protein-bound form to ten volunteers of both sexes and body retention measured by whole-body counting (Rahola et al. 1971, 1973). No difference in absorption between free and protein-bound mercury was found. The whole-body radioactivity measurements revealed three elimination phases with varying half-times. The first phase, dominating during the first 1–2 days after dosing, had a biological half-time of less than 2 days. The second phase, dominating up to 6 days after dosing, had a biological half-time of 2.5 days and constituted 13–38% of the dose. The remaining part of the dose, 1.4–15.6% (mean 7.0), was eliminated according to a biological half-time of about 42 days. The interpretation of these data with regard to absorption is difficult. What is clear is that the 7% (eliminated according to the long biological half-time) had been absorbed. Whether, in addition, a part of the amount eliminated according to the 2.5 days half-time had been absorbed cannot be established with certainty. Part of this phase, as well as the mercury eliminated according to the first phase, undoubtedly represents mercury passing unabsorbed through the gastrointestinal tract.

Elemental mercury introduced into the gastrointestinal tract is absorbed to a very limited extent. Animal experiments (Bornmann et al. 1970) indicate that less than 0.01% of the administered dose is absorbed. An increased blood level of mercury was found in persons who had accidentally ingested large doses (several grams) of metallic mercury (Suzuki & Tanaka 1971).

Specific data on the absorption in human beings of organic mercury compounds other than methyl mercury are not available. For the latter compound the absorption has been shown to be about 95% of the administered dose irrespective of whether the radioactive methyl mercury compound was administered as a salt in water solution (Åberg et al. 1969) or in protein-bound form (Rahola et al. 1971).

III.3.3. PLACENTAL TRANSFER

III.3.3.1. General aspects

In principle, transplacental passage of metals must be distinguished from placental binding as the latter may involve both maternal and fetal tissues. In turn, species differences in placental structure and implantation are likely to influence both the binding and the passage of toxic metals. The hemochorial placental barrier of primates and rats is more easily traversed than the more complex placental barriers found in other species of experimental animals. Placental binding and passage are influenced by gestational age and the availability of the particular metal in maternal blood. Although it is generally recognized that the diffusable fraction of metals in plasma would seem easily transported across the placenta, one must remember that macromolecules can also cross the placental barrier.

Transplacental transfer gives an initial body burden which will be augmented by later environmental exposures. It is prudent to consider fetuses as an especially susceptible population group. If transplacental transfer of a particular metal is high, then fetal exposure may prove a limiting factor upon which exposure standards must be based.

In the USA a systematic collection of cord blood, maternal and fetal tissues has been commenced through the CHESS (Community Health and Environmental Surveillance System) program utilizing standardized tissue collection procedures (Riggan et al. 1972).

III.3.3.2. Specific data

Cadmium. Laboratory studies involving experimental animals have shown a limited passage of cadmium across the placental barrier (literature reviewed by Friberg et al. 1971). The variable penetration observed may be explained by differences in experimental species, challenge compound and dose, route of administration, and gestational age.

Four human studies have shown that cadmium is bound to and crosses the placenta. Dawson et al. (1968, 1969), Piscator (1971) and Finklea et al. (1972) found that cadmium was bound to placental tissue. The passage of cadmium over the placental membrane takes place only to a limited degree, since Henke et al. (1970) found that the liver and kidney of the newborn contained much smaller amounts of cadmium than would be expected in the corresponding organs of the mother. The concentration difference between the maternal and fetal liver can be estimated to amount to a factor exceeding 1,000.

Lead. Placental transfer of lead has been shown (Baumann 1933, Kehoe et al. 1933, Thompsett & Anderson 1935, Horiuchi et al. 1966, Holtzman 1966, Blanchard 1966, Barltrop 1969, Scanlen 1971, Harris & Holley 1972, Haas et al. 1972, Finklea et al. 1972). In a comprehensive survey of human fetal tissues Barltrop (1969) found that transplacental passage became detectable at 12–14 weeks of gestation and that whole body lead increased from that point until parturition. Fetal bone was found to have the highest tissue lead level (80 μg/g). Lower lead levels were found in placenta, heart, kidney, liver and blood.

Haas et al. (1972) reported on 294 sets of maternal and fetal blood samples. Maternal blood samples were 16.9 ± 8.6 μg/100 ml and fetal levels were 15.0 ± 7.9 μg/100 ml. Blood levels in the newborn correlated reasonably well (coefficient of correlation, $r = .57$) with levels in their mothers. Recently four groups of investigators in the United States (Finklea et al. 1972) reported lead levels in maternal – fetal sets of placenta, maternal blood and cord blood. They also found that lead is bound to and passes across the placenta. Fetal blood levels in these studies were generally lower than maternal levels, with maternal erythrocyte levels exhibiting a greater excess than whole blood. Significant regional differences may have been associated with differences in the mothers' environmental lead exposure before the birth of the children.

Mercury. The placental barrier is more effective in preventing the passage of mercuric ion than of elemental mercury. Recently Clarkson et al. (1972) found that after equal exposures of pregnant rats to metallic mercury vapor or mercuric ion, respectively, the fetal uptake of elemental mercury was 10–40 times higher than that of inorganic mercuric mercury, whereas the placental content of mercury after exposure to metallic mercury was only about 40% of that after exposure to mercuric mercury.

Among 44 mothers who gave no history of occupational exposure or high fish intake, Finklea et al. (1972) reported that maternal and cord blood levels were similar to each other, both with a range of 2–20 ng/g.

Alkyl mercury compounds penetrate the placenta easily and intrauterine intoxications have been reported from several nations (Engleson & Herner 1952, Sweden; Harada 1968, Japan; Bakulina 1968, USSR; Snyder 1971, USA; Bakir et al. 1973, Iraq). In a study of women with normal pregnancies and a low or moderate fish intake, methyl mercury levels in fetal blood cells were 30% higher than in maternal erythrocytes (Tejning 1970). This relative excess in fetal blood was confirmed in another study (Suzuki et al. 1971). Mercury levels in the blood cells from the umbilical cord were 31 ng/g (S.D. 22) which were higher than concentrations in maternal blood cells (23 ng/g with a S.D. of 12) but plasma levels were similar (11 and 12 ng/g respectively). Differences in hematocrit and in binding characteristics between fetal and adult hemoglobins may account for the higher blood concentrations found in fetal blood.

III.4. TRANSPORT AND DISTRIBUTION

III.4.1. TRANSPORT AND BINDING IN BLOOD

Both plasma and blood cells can take part in the transport of metal compounds, but generally cell-bound metals do not exchange with other tissues as easily as metals bound to plasma constituents. Partition of a metal between erythrocytes and plasma, which thus may limit its availability for exchange with tissues, varies extensively with different metals and their organometallic compounds as well as with the dose and time after dosing. Thus, e.g. plasma-to-blood cell ratio in man varies from 1.0 (i.e. equal concentration of metal in erythrocytes as in plasma) for normal intake of cadmium, copper, chromium and nickel to only 0.01 for lead (Vostal 1972a). An example of a ratio range among different forms of a metal is shown by mercury: 2.5 for inorganic mercuric mercury after oral absorption (Miettinen 1972), 1.0 for inorganic mercury after industrial exposure to mercury vapor (Lundgren et al. 1967) down to 0.1–0.2 for methyl mercury in man (Birke et al. 1972). For the same chemical form of metal the ratio changes also in various animal species, e.g. data for methyl mercury range from 0.002–0.004 in rat and chicken to 0.03–0.05 in guinea pig, dog and Rhesus monkey, and 0.1–0.2 in swine, rabbit, mouse (Vostal 1972b) and man (Birke et al. 1972).

Many constituents of erythrocytes may participate in metal binding. Metals may thus be bound to hemoglobin and other specific metalloproteins or to erythrocyte membrane. A specific chemical form of a metal usually displays a specific distribution pattern among these constituents.

Although a role of erythrocytes for a direct exchange of metals between circulating metal in blood and between tissues cannot be excluded, plasma is generally considered as the main mediating pathway for transport of metals. Erythrocytes should therefore be looked upon mainly as one of many compartments in the body from which there is a continuous exchange of metals with plasma.

The binding of metals to plasma proteins is related to the occurrence of ligands and to the magnitude of the stability constants for the metal ligand bond. The stability of the metal-protein bond varies with the type of protein as well as the metal involved, and in general, three different forms of existence of metal in plasma can be assumed:

(1) Metal firmly bound to a specific plasma metalloprotein demonstrated so far to exist only for a few metals; the metal-protein bond is so firm that the metal is not removed by usual isolation and purification procedures. Protein can be isolated and the structure of the metal-protein bond studied in detail. Release of the metal from the bond *in vivo* can therefore occur only in the presence of an excess of other ligands with high affinity for metal, by exchange with metal with higher affinity, or when the protein is catabolized.

(2) Metals bound to various fractions of plasma-proteins, e.g. albumin. Metal is bound loosely to protein and dissociates readily.

(3) Metal bound to low-molecular weight plasma components; this fraction will probably behave differently for individual metals in regard to diffusibility or ultrafiltrability, but is usually included in what is called the "diffusible" fraction, consisting of other fractions of metal bound to various forms of diffusible organic ligands as e.g. amino acids, etc. Theoretically, based on the mass action law, a minute amount of metal should be in ionized form to sustain the equilibrium with "bound" fractions of metal.

The low-molecular weight or "diffusible" fraction of metal in plasma is of fundamental interest for transfer of metal to and from various organs and for excretory mechanisms. This fraction has not yet been quantitated for many metals, partly because of methodological difficulties in separating the very small metal concentrations at the steady state dominated largely by the protein-bound fraction and partly because detection limits of analytical methods are such that it is often impossible to detect these low concentrations under conditions of "normal exposure".

Small amounts of elemental mercury have been found to exist physically dissolved in plasma for a short time after exposure to metallic mercury vapor (Magos 1967).

III.4.2. DISTRIBUTION

III.4.2.1. General aspects

Different organs manifest striking variations in the fraction of the total body burden of any metal that each contains. Factors that determine the transfer of a metal from plasma to a particular organ are complex and their quantitative aspects are not fully understood.

However, the following factors may be considered to be of significance:

(1) Fraction of the metal in plasma that is in the "diffusible" form and the rate of organ perfusion (*in vivo*).

(2) The permeability of cell membranes for the metal in the forms that occur in plasma.

(3) The availability and turnover rate of suitable membrane and intracellular ligands for the metal.

A simplified model for the exchange of metal between the circulating plasma and organs, shown in Fig. 16, considers the relationships among five compartments: the "non-diffusible" plasma protein-bound fraction, the plasma "diffusible" fraction, the interstitial (extracellular) "diffusible" fraction, the intracellular "diffusible" fraction, and the intracellular "non-diffusible" protein-bound fraction.

As stated above, the plasma "diffusible" fraction is thought to be of prime importance in the transfer of metals between intravascular extracellular and intracellular compartments. The protein-bound fractions in plasma and in the cells constitute depots which are supposed to equilibrate continuously with the more "diffusible" fractions.

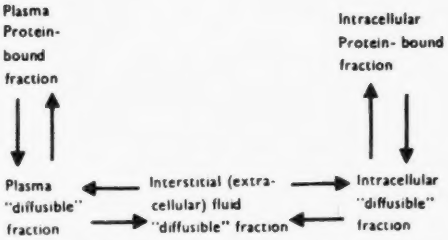
Plasma Protein-bound fraction
Intracellular Protein-bound fraction
Plasma "diffusible" fraction
Interstitial (extracellular) fluid "diffusible" fraction
Intracellular "diffusible" fraction

Figure 16. Model for the exchange of metal between blood and other tissues.

Binding of a metal with an intravascular or intracellular ligand disturbs the equilibrium and creates a concentration gradient that favors transfer of metal to that depot. Any change in the availability of ligands in the plasma or in the cells may similarly shift the equilibration in either direction and result in the movement of metal.

This simplified scheme for the general type of exchange between plasma and tissue certainly does not consider the specific properties of diversified cell systems within an organ, as e.g. the reticulo-endothelial system in the liver, or of organs with diversified functions such as e.g. the kidney. In the kidney, tubular cells may accumulate metals from tubular fluid in addition to direct uptake from plasma. It must be furthermore assumed that many other factors such as e.g. catabolism of plasma proteins, may also contribute to the deposition of metals in some organs. The distribution of the metal may be influenced by the possibility that metals can get a "free ride" on intra- and extracellular carriers of other metals. Organs such as the brain and the testes may be protected by "barriers" through which some metals can be transferred only to a limited extent.

The amount of metal that is accumulated in the cells of an organ is obviously the difference between the amount that has been transferred into the cells of the organ and the amount that has been transferred out. The same amount can accumulate in the cells of an organ when the rates of transfer in and out are high as when the rates of transfer in and out are low.

The level of exchange rate is, however, a limiting factor in achieving the steady state. Steady state may be delayed with low levels of exchange rates for a long period of time. This should be considered especially in cases in which the levels in the organ are extrapolated from stationary levels in plasma, although steady state might not yet have been achieved.

III.4.2.2. Consideration on the penetration of metals into the brain

III.4.2.2.1. General aspects

Although it is evident from the foregoing discussion that specific transfer systems for metals may exist for several organs in the body, the brain has been chosen for special consideration here; accumulation of metals in the kidney will be discussed to some extent in the section on renal excretion.

The central nervous system, usually considered as a special compartment separated from blood by the so-called blood-brain barrier, often shows a slow uptake of metals, probably due to a low transfer rate through this barrier when met by a metal compound circulating in the intravascular compartment of the brain.

Penetration rates depend on the form in which the metal or organometallic compound enters the brain. Thus e.g. it has been shown experimentally that the uptake of mercuric ion in the mouse brain is facilitated by complexing with dimercaptopropanol (Berlin & Lewander 1965). In general, little is known about metal transport and deposition in brain. On the basis of limited data, the turnover of metal deposits in brain appears to be slow for a number of metals.

As there are differences in structure and metabolism between adult and infant brain, differences in metal uptake and turnover rate can be postulated. Epidemiological data on incidence of intoxication and signs of poisoning in relation to exposure to methyl mercury give some support to such a postulate.

III.4.2.2.2 Specific data

Cadmium. Cadmium does not easily penetrate into the brain (Berlin & Ullberg 1963b, Nordberg & Nishiyama 1972) and only traces were found in the brain of mice after a single intravenous injection of ^{109}Cd. Human data are scarce, but it can be mentioned that in a case of excessive exposure to cadmium where the concentration of cadmium in liver was 94 ppm wet weight, the concentration in brain was 0.6 ppm (Ishizaki et al. 1970).

Lead. Lead passes into the brain but does not accumulate there to any great extent (Schroeder & Tipton 1968, Barry & Mossman 1970). The local distribution of lead in the brain may be of more importance than the mere passage from blood to brain. The concentration of lead has been found to be higher in cortical grey matter and basal ganglia than it is in cortical white matter in cases of lead intoxication (Klein et al. 1970). Horiuchi (1970) found 23 μg/100 g (wet weight) as the average concentration in the cerebrospinal fluid of 6 normal Japanese adults. These quantities are similar to those found in the cerebrum and cerebellum and are consistent with brain lead levels reported from various countries (Schroeder & Tipton 1968).

Nonpolar organolead compounds penetrate more rapidly into brain, as well as other membranes, than do inorganic lead compounds. This penetration will often be as the partially metabolized material, i.e. triethyl lead compounds following administration of tetraethyl lead (Cremer 1965). There are some differences in brain levels for different organolead compounds (Schepers 1964). Although the brain is the critical organ for organolead compounds, the concentrations of lead in the brain do not highly exceed those in other tissues (Cassels & Dodds 1946).

Mercury. Animal data published by Friberg (1956) showed that the brain-to-blood ratio increased during five weeks of exposure to inorganic mercury, and Berlin & Ullberg (1963a) found the same phenomenon after a single injection in mice. Similarly, Swensson & Ulfvarson (1968) found that brain levels of mercury were several times higher than those in the blood 10 or 20 days after single injection of mercuric chloride in poultry. Moreover, after exposures to elemental mercury, the brain reaches levels several fold higher than after similar doses of mercuric salts (Berlin et al. 1966, Magos 1967, 1968, Nordberg & Serenius 1969). Mercury levels in brain, several times higher than those in liver and other organs except kidney, were reported in miners with long-term exposures to high concentrations of mercury vapor even several years after cessation of exposure (Takahata et al. 1970, Watanabe 1971). Large differences among various parts of the brain have been demonstrated with regard to mercury concentration after exposure to mercury vapor as well as after injection of mercury salt (Nordberg & Serenius 1969, Berlin et al. 1969b, Cassano et al. 1969).

After administration of methyl mercury compounds, mercury levels in the brain are higher than after administration of inorganic mercury compounds or aryl mercury compounds (Berlin 1963). Åberg et al. (1969) found approximately 10% of total body burden of perorally administered methyl mercury in the posterior region of the head of human volunteers.

Data on the distribution of mercury in various species of animals were recently collected and considerable differences in distribution ratios between blood and brain discovered (Berglund et al. 1971). It was shown that the ratio between the levels in blood and brain is approximately 10–20 in rats, 1.0 in mice, 0.5 in dogs and swine and 0.1–0.2 in primates. Wide interspecies differences in plasma to red blood cell ratios (Vostal 1972b) may account for these variations in the blood-to-brain ratios since the plasma-to-brain ratios are very similar in the various species.

The distribution of mercury among different parts of the central nervous system after administration of methyl mercury has been studied in animal species. The data show differences in mercury concentrations among different parts and structures of the brain (Berlin 1963, Nordberg et al. 1971b, review by Berglund et al. 1971).

III.5. EXCRETION

III.5.1. GASTROINTESTINAL EXCRETION

III.5.1.1. General aspects

The amount of metal found in feces derives from the fraction of ingested material that passes unabsorbed through the gastrointestinal tract and the net gastrointestinal excretion. Net gastrointestinal excretion consists of metal which has been excreted into the lumen of the gut from the gastrointestinal tract (total gastrointestinal excretion) minus the amount of metal reabsorbed. A scheme illustrating the various routes is seen in Fig. 3.

The relative importance of gastrointestinal excretion varies from metal to metal and is, furthermore, dependent on several factors, such as the route of absorption (Dutkiewicz, in Dukes & Friberg 1971, Nordberg & Skerfving 1972), type of exposure (acute or prolonged), and a time dependent organ distribution.

Gastrointestinal excretion takes place by a number of routes. The most important of these are (1) active secretion or simple passive loss of metal with the secretion from various glands into the alimentary tract, i.e. salivary glands, pancreas, and glands located in the intestinal epithelium (Berlin, in Dukes & Friberg 1971), (2) loss by the shedding of epithelial cells, and (3) biliary excretion.

Different parts of the gastrointestinal tract have different excretory capacities depending on the cellular structure of the intestinal wall. Excretion, furthermore, depends on the concentration of the metal in plasma and the organs excreting their contents into the gut. Present data do not allow definite conclusions as to the mechanism(s) of gastrointestinal secretion of metals, i.e. whether mediated by specific excretory mechanisms or transferred passively. There are some data suggesting a specific excretion process for metals in the cells of the gastrointestinal mucosa. Berlin & Ullberg (1963a, b) found high amounts of mercury and cadmium in various parts of the gastrointestinal mucous lining, both within the cells and outside them as a halo adjacent to the luminal border. Although these data can be explained by passive transfer, they could also indicate a specific secretory process for metals in those cells. Such a secretory function has been postulated for the cells of Paneth (Halbhuber et al. 1970), but their relative importance for total gastrointestinal excretion of metals is likely to be low.

The excretion by intestinal cell shed is often of major importance for the total gastrointestinal excretion. The cells in the gastrointestinal mucosa have a high turnover. Since part of their metal content is derived from circulation (the rest being taken from the intestinal content), significant amounts of metal are removed by this mechanism. The mechanism is particularly important when plasma concentrations are high, and thus the major source of metal for the cell will be circulatory and not uptake from intestinal contents. This excretion route appears to be particularly important for methyl mercury (Norseth & Clarkson 1971).

Elimination of lead and mercury has also been related to gastrointestinal catabolism of proteins (Witschi 1964, 1965). This mechanism may, however, account for only a small amount of the excretion because of the low amount of plasma proteins normally catabolized in the intestinal tract (Kerr et al. 1967). The part accounted for by this mechanism would also have to pass through the gastrointestinal mucous lining and thus will be included in one of the above-mentioned mechanisms.

Biliary excretion may be the most important pathway contributing to gastrointestinal excretion. Plasma fractions of several metals bound to proteins have been shown to be associated with excretion of the metal into the bile (Holmberg 1961, Gaballah et al. 1965). This is in accordance with the hypothesis that only compounds with a molecular weight higher than about 400 are preferentially excreted in bile (Abdel Aziz et al. 1971). In accord with this, metals and organometallic compounds in the bile are bound to proteins or organic ligands with relatively high molecular weight (Norseth & Clarkson 1971, Cikrt 1972). However, it has not been possible to assess whether this binding is a true part of the excretion mechanism or a result of a secondary complexing to some substance in the bile. The nature of the complexing agent has been shown to be important for the degree of reabsorption (Norseth & Clarkson 1971) of metal primarily excreted to the gut. In this way the nature of the complex formed will be of great importance for net gastrointestinal excretion of metals. Reabsorption, presumably in the form of such complexes, has been shown to take place for methyl mercury and cadmium (Norseth & Clarkson 1971, Caujolle et al. 1971).

Differences among species have been described for the extent of biliary excretion in relation to other routes of excretion of metals (Vostal 1972b). Caution should be exercised when applying conclusions from animal data to man, especially concerning the quantitative importance of biliary excretion. Several metals which have been demonstrated in the bile of experimental animals may not necessarily be found to the same extent in human bile. The role of biliary excretion and reabsorption in gastrointestinal transport in man therefore remains uncertain.

III.5.1.2. Specific data

No human data on total gastrointestinal excretion exist. Studies have been made only on net gastrointestinal excretion or content in feces of the metal in question.

Cadmium. Rahola et al. (1971) studied 5 male volunteer subjects (ages 19–50) who had ingested ^{115m}Cd mixed with calf kidney proteins (there are data from this study in section 3.2.2). They estimated that the net gastrointestinal excretion of the amount retained in the body was less than 0.1% per day.

By animal experiments cadmium has been demonstrated in the intestinal mucosal lining and in pancreas, indicating a role of these organs in the slow elimination of this metal (Berlin & Ullberg 1963b, Nordberg & Nishiyama 1972).

Biliary excretion of cadmium is found in the rat (Caujolle et al. 1971) and cadmium has also been found in the bile of human autopsy cases (Smith et al. 1955, Tsuchiya et al. 1972b).

Lead. Available evidence suggests that gastrointestinal excretion of lead may not be as quantitatively important as those of cadmium and mercury (Clarkson in Dukes & Friberg 1971). A review of balance studies by Kehoe et al. (1943) suggests that clearance of unabsorbed lead from the gastrointestinal tract accounts for most of the fecal excretion of lead. Hursh & Suomela (1967) measured gastrointestinal excretion of lead in two human volunteers following a single intravenous administration of ^{212}Pb. Less than 0.5% was found in feces during the first 48 hours after the injection.

Excretion of lead in bile was postulated many years ago by Cantarow & Trumper (1944) but supporting data are scarce. Castellino et al. (1966) recovered 8.4% of intravenously injected ^{210}Pb in the bile of rats in 50 hours, and Cikrt (1972) reported 6.7% in 24 hours. In a similar experiment using sheep, Blaxter & Cowie (1946) recovered about 6.0% of an intravenously injected dose of lead salt in bile over a 6-day period. More recently Gage & Litchfield (1968) found in rats that slight increases in dietary lead (2; 6 and 20 ppm) were not associated with detectable increase of urinary lead, but as little as 6 ppm increased the bile content of lead.

Mercury. After a single peroral dose of mercuric mercury was given to human volunteers, less than 1% of the dose per day was recorded as net gastrointestinal excretion after the initial passage of unabsorbed material (Rahola et al. 1971, in press).

Animal experiments indicate that excretion of inorganic mercury by the gastrointestinal tract is dependent on dose and time of the exposure. The fecal route of excretion dominates soon after exposure. This excretion route is also favored when high doses are given. (Prickett et al. 1950, Friberg 1956, Rothstein & Hayes 1960, Ulfvarson 1962, Cember 1962, Phillips & Cember 1969, review by Nordberg & Skerfving 1972). After such inhalation exposure, the oxidation of metallic mercury to mercuric mercury is fast enough to prevent the diffusion of appreciable quantities of mercury into the gastrointestinal tract. Accordingly, such diffusion will be of no consequence for total excretion.

In human beings, methyl mercury is mainly excreted in feces and the proportion does not vary with the dose levels or exposure time, 90% of the total excretion taking place in feces after a single dose (Åberg et al. 1969, Rahola et al. 1971). Mercury was also found in the mucosal lining of mice after methyl mercury exposure (Berlin & Ullberg 1963a) and intestinal cell shed was suggested to be a major source of fecal metal in rats (Norseth & Clarkson 1971).

No experimental data on gastrointestinal excretion from humans exist for other organic mercury compounds. Animal experiments indicate that gastrointestinal excretion varies with the exposure pattern and levels of exposure (review by Nordberg & Skerfving 1972).

Biliary excretion of both inorganic and organic mercury compounds has been demonstrated in the rat (Norseth & Clarkson 1971, Cikrt 1972). The significance of this excretion for net gastrointestinal excretion is uncertain because of a possible reabsorption (Norseth & Clarkson 1971). Furthermore, conclusions with regard to other species are impossible because of interspecies differences in biliary excretion of both inorganic and organic forms of mercury (Vostal 1972b).

III.5.2. RENAL EXCRETION

III.5.2.1. General aspects

The highly complex physicochemical state of metals in blood makes it difficult to use classical physiological methods for exploring how the metals are handled by the kidney. Routine methods for the calculation of renal clearance may be used only for the portion of metal which is present in plasma in "diffusible" form and can pass through the glomerular membrane or tubular cells.

The glomerular ultrafiltrate contains metals bound to compounds of varying molecular sizes and properties, from low molecular weight proteins to simple anions. Glomerular capillaries behave as though they were perforated by pores of a size just adequate to permit the passage even of plasma albumin molecules (Pappenheimer 1955). However, it is known that only a minute fraction of plasma albumin passes the membrane. The combined effects of steric and electric hindrance and of viscous drag (Wallenius 1954) restrict filtration rates of the major amounts of plasma proteins. The proportion of such proteins with large molecular weight that passes the glomerular membrane is only a small fraction of the filtration of plasma water. Substances of relatively low molecular weight, e.g. purified tuberculin protein (mol. wt. 14,500) or inulin (mol. wt. 5,000) are excreted into the tubular lumen more readily (Bott & Richards 1941). Metals bound to low molecular weight proteins may consequently be cleared from the plasma by glomerular filtration mechanisms.

The plasma filtrable fraction and the renal clearance have been determined for copper (Walshe 1961), nickel (Hendel & Sunderman 1972), radium (Hursh et al. 1960), zinc (Prasad 1966) and chromium (Collins et al. 1961, review of all metals: Vostal 1972a). Values for their renal clearance were lower than the glomerular filtration rate and indicated that tubular reabsorption was involved in the renal excretory mechanisms of all studied metals. Analytical inaccessibility of plasma filtrable fractions of lead and mercury prevented similar direct analysis of renal excretion of these metals. Calculations by indirect methods (Vostal 1972a) suggest that tubular mechanisms may also participate in the renal excretion of these metals.

The plasma filtrable fraction may be influenced by the presence of increased concentrations of some normal plasma components with the ability to bind metal ions. As examples may be mentioned that sodium bicarbonate increases the filtrable fraction and excretion of uranium (Neuman 1949). Cysteine enhances the passage of mercury and organomercurials into the tubular urine (Clarkson & Vostal 1972), and histidine and other amino acids may influence the amount of copper and nickel in plasma ultrafiltrate (Sarkar & Kruck 1966, von Soestbergen & Sunderman 1972).

Since many of these compounds have their own cellular transport system involving tubular secretion or reabsorption, their participation in renal handling of metals cannot be excluded. Metal ions can get a "free ride" and follow the renal pathways of the organic moiety. This obviously would modify the mechanism of renal handling and clearance of metals, as discussed for high exposures to lead (Vostal 1963).

Changes in urinary pH are followed by acute effects on the urinary excretion of uranium (Neuman 1949) and lead (Webster 1941, Vostal 1966), which suggests the existence of tubular reabsorption of these metals.

Any reabsorptive process for a metal during its passage through the renal tubule results in its entrance into renal tubular cells and may lead to accumulation of the metal in renal tissue if the metal is not transported further into the peritubular capillary blood. It is not known in any detail to what extent the existence and effectiveness of these tubular reabsorptive processes may depend on factors such as changes in urine pH, metal charge, competition with other compounds, etc.

That fraction of metal which has not been reabsorbed is excreted and usually represents a major part of the urinary excretion of metal. In addition, shedding of tubular cells containing the metal may contribute to excretion. Thus, specific avidity of the renal tissue for a metal may introduce a variable factor in the process of renal excretion of metals. The possibility exists that metals may be added to or extracted from the urine during their further passage along the urinary tract, but little is known about such a process.

The mechanisms for accumulation of metals in the renal tissue are largely unknown. Some metals are present in the kidney tubules in the form of specific tissue-metalloproteins (e.g. metallothionein) with binding ability for cadmium, zinc, copper and mercury (Kägi & Vallee 1960, 1961, reviews by Friberg et al. 1971, Piotrowski et al. 1972). A lead protein complex has also been identified in the intranuclear inclusion bodies in renal tubular lining cells (Richter et al. 1968, Goyer et al. 1970, Goyer 1972). These proteins mainly constitute a storage pool for metals; when the capacity of available intracellular metalloproteins is exceeded, redistribution of the metal may occur.

III.5.2.2. Specific data

Cadmium. The low levels of cadmium in plasma (review by Friberg et al. 1971) and its binding mainly to proteins with a molecular size similar to or larger than albumin (Nordberg et al. 1971a) make it difficult to study the renal excretory mechanisms for cadmium, especially under conditions of low or moderate exposure. Piscator (1964) and Friberg et al. (1971) introduced a model for renal accumulation and excretion of cadmium implying a role of metallothionein. The participation of metallothionein-bound cadmium in urinary excretion has been supported by animal experiments with exposures sufficient to induce proteinuria (Nordberg & Piscator 1972).

Lead. The analytical inaccessibility of the small plasma concentrations is the reason for the lack of knowledge on the quantitative aspects of the mechanisms responsible for urinary lead excretion under conditions of "normal" exposure. By an indirect method, Vostal (1963) postulated the existence of tubular reabsorption mechanisms for lead in acutely exposed subjects with high blood levels of lead (more than 100 μg/100 ml), whereas the role of these mechanisms in persons and animals with lower levels in the blood was considered insignificant. Further evidence for the participation of tubular reabsorption has been discussed by Vostal (1972a).

Mercury. Recent reviews on the renal excretory mechanisms of mercury concluded that despite the presence of a minute diffusible fraction in plasma, glomerular mechanisms do not completely explain urinary excretion of mercury and its compounds (Vostal 1969, Nordberg & Skerfving 1972, Clarkson 1972).

Experiments in rats (Clarkson & Magos 1970) indicate a peritubular transfer of mercuric ion from blood into renal tubules. Transport via the tubular wall into urine has been shown in avian kidney (Vostal & Heller 1968). This excretion route might also be important in humans (Vostal 1972a). There might be an exposure-dependent change in the mechanism for mercury excretion, judging from variations in the proportion of different forms of mercury in workers' urine (Henderson et al. 1972).

III.5.2.3. Renal excretion in relation to functional impairment of organs

As the renal handling of metals is dependent on glomerular function, tubular function and the state of the renal tissue, any change in one of these functions may cause changes in distribution or excretion of metals. Generally, any renal disease with high proteinuria may lead to increased excretion of metals bound to high molecular weight proteins and eventually lead to depletion of body stores of metals. Renal disease with decreased filtration may lead to higher blood levels of certain metals bound to low molecular weight protein or other low molecular weight compounds. Congenital or acquired tubular dysfunction will cause a decreased reabsorption and increased excretion of those metals ordinarily reabsorbed at different sites in the tubules. Furthermore, there may be an excretion of metal through the exfoliation of tubular cells.

A change in excretion of metals may also be due to metabolic changes, as shown for mercury (Clarkson & Magos 1967, Magos & Stoytchev 1969). Similarly, changes in the acid-base balance are likely to change excretion and reabsorption rates. It is known that alcohol will cause an increased excretion of some essential metals, possibly due to liver impairment (review by Piscator 1972b).

There is reason to believe that accumulation of some of the metals in the kidney can give rise to changes in renal function and thus in the renal excretion of the metal itself. Only in the case of cadmium, however, has this phenomenon been investigated in sufficient depth. Since normally the amounts of cadmium excreted in urine are very low, there is probably a very efficient tubular reabsorption of the cadmium-containing protein (Friberg et al. 1971). Animal experiments (Friberg 1952, Axelsson & Piscator 1966, Nordberg & Piscator 1972) have shown that when renal damage occurs, there is a sudden increase in cadmium excretion simultaneously with the appearance of proteinuria. Results from investigations on workers exposed to cadmium indicate similar effects in man (Piscator 1972a). The changes in the turnover of cadmium may lead to changes in distribution so that in advanced renal damage, urinary losses of cadmium will increase and eventually the renal concentration of cadmium will approach the "normal" range (review by Friberg et al. 1971).

Hemolysis may increase the amount of filtrable plasma cadmium as indicated by experiments on rabbits (Piscator 1963). Other extra-renal factors that could be of importance for the excretion of cadmium are infection or trauma. It was found by Bonnell et al. (1959) that cadmium excretion was increased in workers with pneumonia or pneumothorax. Possible mechanisms responsible for the mobilization of cadmium probably involve sudden release of accumulated metal in the lung tissue.

III.5.3. MAMMARY GLAND EXCRETION

III.5.3.1. General aspects

Exposure of the newborn through milk will constitute a hazard, especially in cases in which the initial body burden is already raised due to transplacental transfer. This hazard sometimes exists even when the initial body burden is negligible (Bakir et al. 1973). The processes through which the metals under consideration are excreted through the mammary glands are not known. The apocrinic type of secretion typical of mammary glands will, however, provide a possibility for transfer to the milk of all metals with a tendency to accumulate in mammary gland cells.

III.5.3.2. Specific date

Cadmium. Berlin & Ullberg (1963b), detected cadmium in the mammary glands of mice injected with ^{109}Cd. Miller et al. (1967) exposed cows to cadmium by daily oral doses of 3 g for 2 weeks. The cadmium concentration in milk never exceeded 0.1 ppm, and it was calculated that less than 0.02 % of the dose was excreted via milk. Lucis et al. (1971) gave nursing rats injections of ^{109}Cd and found that cadmium was retained in mammary tissue and that low quantities were found in milk. Lucis et al. (1972) studied the soluble proteins from the mammary gland of cadmium-exposed pregnant rats and found that cadmium was bound to high molecular weight proteins.

Murthy & Rhea (1971) found that human milk in the USA contained on the average 0.019 ppm of cadmium, which is higher than the range of 0.001–0.010 found in cow's milk in different countries (Friberg et al. 1971).

The animal data indicate that although cadmium is taken up in the mammary gland tissue, it is excreted only in small amounts via milk. There is a lack of human data.

Lead. Excretion via milk seems to be of importance, and as excretion will increase when blood levels increase, exposure via milk may be of importance in children of lead-exposed women, especially if there has also been prenatal exposure.

Hammond & Aronson (1964) found that the lead concentration in milk from normal cows was 0.009 ppm on the average, whereas in cows exposed to lead, concentrations of 0.05–0.27 ppm were found. The lead concentrations in milk were correlated to lead concentrations in blood. A relationship between lead concentrations in blood and milk of cows can also be found from the data of Donovan et al. (1969).

Lead poisoning can be produced in suckling rats or mice by exposing the mothers to lead (Pentschew & Garro 1966, Rosenblum & Johnson 1968).

The average lead concentration in human milk was found to be 0.012 ppm in the USA (Murthy & Rhea 1971) and from trace to 0.12 ppm in three samples from Japan (Horiuchi 1970). There are data that indicate that in industrialized areas human milk may have higher concentrations than cow's milk or formula milk (Noirfalise et al. 1967).

Mercury. After injection of radioactive mercury chloride in mice, mercury was found in the mammary glands (Berlin & Ullberg 1963a). In lactating guinea pigs given a single intraperitoneal injection of methyl mercury, the mercury levels in milk were generally below 2 % of the whole blood levels (Trenholm et al. 1971). Investigations on lactating women who had been exposed to methyl mercury in fish (Skerfving & Westöö 1972) or in bread (Bakır et al. 1973) revealed that the total mercury concentrations in milk averaged about 5 % of the total mercury concentrations in blood (Skerfving & Westöö 1972) or the organic mercury concentrations in milk averaged about 3 % of the mean organic mercury concentrations in blood (Bakir et al. 1973).

III.6. ACCUMULATION AND RETENTION IN CRITICAL ORGANS

III.6.1. GENERAL ASPECTS

III.6.1.1. Uptake and retention

The critical organ accumulates metal when uptake exceeds elimination. A steady state is reached when uptake and elimination are equal. Provided that the metal movement is determined by concentration gradients, a slow uptake will result in a longer time for reaching steady state. With a low elimination rate the metal will be retained for a long time even if uptake is stopped.

III.6.1.2. Biological half-time

A common way to express the elimination rate is in terms of biological half-time, which is a measurement of the elimination rate when an elimination phase succeeds the steady-state condition. "Biological half-time" is only meaningful when the elimination can be approximated with reasonable accuracy to a single exponential first order function. This is fulfilled when the metal movement from the organ is mainly dependent on concentration gradient. The concentration in the organ at any time then can be expressed by the following equation:

$$C = C_0 \times e^{-bt} \qquad (1)$$

C = concentration in the organ at time t.
C_0 = concentration in the organ at time 0.
b = elimination constant
t = time

The relation between the elimination constant and the biological half-time is as follows:

$$T = \frac{\ln 2}{b} \qquad (2)$$

T = biological half-time
ln 2 = logarithm for 2 = 0.693

The exponential function is not always representative of the elimination of toxic metals; therefore the equations should be used with caution.

III.6.1.3. Concentration in critical organ at continuous exposure

Of importance for toxicological evaluation is the highest concentration reached in the critical organ at continuous exposure. The dominant factor determining that concentration, in addition to the exposure, is the rate of elimination of the metal from the body and in particular from the critical organ. A low elimination rate will cause accumulation of metal in the critical organ even at low exposure, whereas at rapid elimination rate the exposure level will be the stronger factor.

To predict the concentration in the critical organ at constant exposure and at a certain time requires a mathematical model based on intake level and elimination rate. A simple model based on the assumptions that (1) metal movement is linearly dependent on concentration gradient, (2) the rate of uptake of the metal is faster than the elimination rate, and (3) a constant fraction of the intake is taken up by the organ—gives the following expression for the concentration in the critical organ:

$$A = \frac{a}{b}(1 - e^{-bt}) \qquad (3)$$

A = accumulated amount
a = fraction of daily intake taken up by the organ
b = elimination constant
t = time of exposure

At steady state:

$$A = \frac{a}{b}$$

This simple model has been extensively used by ICRP for calculation of accumulation of radionuclides. These calculations have constituted a major step fofward for predicting risks at exposure.

Recently the simple model has also found use for calculations of risks of chemical toxicity. An example of a metal compound for which the model is applicable is methyl mercury. However as stated above, this model is based on the assumption that intake and absorption are constant. If either one of these changes systematically by age, period in time, etc., the equation must be adapted, as attempted by

Tsuchiya & Sugita (1971), Kjellström et al. (1971), and Kjellström & Friberg (1972). For most metals, the assumption that all of their movement in the body is dependent only on concentration gradient is an oversimplification. Active transport or metabolic processes are often involved in metal turnovers. Consequently it has been recognized and also demonstrated experimentally that generally this simple model does not give a sufficiently accurate description of the concentration in the critical organ.

The application of the model requires knowledge about intake (a) and the excretion constant (b) or biological half-time ln $2/b$. For metals with long biological half-times, the concentration in critical organs (allowing calculation of the amount (A) accumulated in the organ) has been determined at autopsy in a cross-sectional sample of human beings and the intake (a) during lifetime for each case has been calculated. Though this method is often the only way to obtain human data, it is subject to difficulties and sources of error. The fundamental prerequisites (outlined above in connection with equation (3)) for such calculations must be fulfilled. Such is not the case: (1) if there is time-dependent variation in absorption and distribution or in exposure (discussed above and by Kjellström & Friberg (1972)); (2) if the biological half-time in the population is not constant. There is reason to believe that the biological half-times of particular metals in the body may vary depending on dose, sex, age and physiological characteristics of a person. One obvious difficulty when using autopsy material is that the cause of death may influence the concentration in the critical organ at autopsy.

In cases of biological half-times lasting over decades, changes in exposure resulting from changes in the general environment (due to altered technology), in food consumption or smoking habits of the population may be of great importance. If such changes in exposure cannot be ruled out or corrected for, it will not be possible to calculate biological half-times (Kjellström & Friberg 1972).

A possible way to get information concerning the importance of past changes in exposure, and to some extent other influencing factors as well, is by periodic replication of autopsy studies which measure metal residues in critical organs. Such studies will allow an empirical description of how metal levels change over time in each cohort of the population. That is, each cross-sectional replicate will add another point to all but the oldest cohort curve. One can then compare the resulting cohort curves and make inferences which could be helpful in constructing models to assess biological half-time and in estimating secular trends in human exposure.

III.6.2. SPECIFIC DATA

In spite of the difficulties in adequately describing the accumulation in critical organs of some metals, an attempt will here be made to provide information on the use of models for cadmium, lead and mercury accumulation in tissues and numerical values for fractions to critical organs and biological half-times (data on absorption will be found in section 3.2.2).

Cadmium. A simple model implying that a constant fraction of absorbed cadmium is directly transferred to the kidney may not be adequate to define renal accumulation of cadmium (Nordberg 1972c) since a part of the transfer probably occurs slowly via the liver. However, at present no other models have been used.

Using the simple model, biological half-times for the kidney between 17.6 years (Tsuchiya & Sugita 1971) and 33 years (Kjellström et al. 1971) have been estimated using available information on the accumulation of cadmium in cross-sectional samples of Japanese, US and Swedish populations.

The fraction of whole body cadmium that will be deposited in the kidney varies depending on the time after administration (according to animal data compiled by Friberg et al. (1971). Autopsy material from a normal population at age 50 (Friberg et al. 1971) indicates that ⅓ of the body burden is located in the kidneys. However, the fraction of the body burden that is found in the kidney is smaller than ⅓ at higher exposure (industrial) (Friberg et al. 1971, Nordberg 1972c).

Using model (3) and correcting it for systematic changes in caloric intake and kidney weight during lifetime, it has been calculated (Kjellström et al. 1971, Kjellström & Friberg 1972) that the cadmium concentration in kidney cortex at 50 years of age would be 50 ppm following a daily intake of 24 ng/calorie (65 μg/day for an adult) assuming about 1.7% (⅓ of 5%) deposition of body intake in the kidney.

Lead. The accumulation of lead has been evaluated by analysis of human tissues obtained at autopsy (Nusbaum et al. 1965, Schroeder & Tipton 1968, Barry & Mossman 1970, Horiuchi 1970). The studies agree as to the general increase of lead in bone and aorta with age. However, the lead in bone was found to (1) increase linearly throughout life (2) reach a plateau after the third decade or reach a plateau from the fourth through the seventh decades but decrease thereafter. A complete analysis of the statistical significance of these various trends has not been performed and a definite interpretation at the moment is impossible.

All of these autopsy studies lack information about previous exposure and excretion rates. It is not clear if the scatter of the data represents mostly biological or environmental variation. These data, therefore, cannot be used to confirm theoretical models of retention of lead.

Indirect estimates of retention of lead have been made by balance studies in human subjects (Kehoe 1961, Horiuchi 1970). Although these studies have been made on relatively few individuals, they have been conducted for prolonged periods of time. They are limited to healthy adults receiving different doses of lead during their adult lives. These studies provide the best estimates of total body retention for specific levels of intake as well as the rate of elimination from the body after the administration of lead was terminated. Only limited attempts have been made to fit the data from these studies to a mathematical model (Sterling et al. 1964).

Knelson et al. (1972) studied human beings continuously exposed to a mean level of atmospheric lead oxide of 10.9 μg/m^3 or 3.5 μg/m^3 for a period of 4 months. Data derived from their studies, namely blood and urine lead, were used to develop a two-compartment mathematical model describing lead uptake and excretion kinetics for humans exposed to atmospheric lead.

The major problem with establishing a mathematical model for lead absorption or lead retention is the strong affinity of lead for bone. The estimates of half-times for lead in bone have varied from 64 days in the spine of rats (Torvik et al. 1972) to 7,500 days for the skeleton of a dog (Fisher 1969). The half-time varies with different bones, presumably due to the relative proportion of cortical to trabecular bone. The ICRP has used 10 years as the biological half-time for lead in bones of humans (ICRP publication 2, 1959).

Prerovska & Teisinger (1970) have also indicated that there are at least two compartments for lead in bone. Hammond & Aronson (1967) also proposed an additional compartment consisting of lead firmly bound to soft tissue. This compartment is consistent with the observations by Goyer (1972) that lead binds firmly with nuclear proteins in rats.

Studies in animals by Bolanowska et al. (1967) and Bolanowska & Piotrowski (1968, 1969), have provided mathematical models for excretion of lead based upon an exponential model with a four-compartment system:

(1) a rapid-exchange compartment (blood and internal organs),
(2) the skin and muscles,
(3) the exchangeable part of lead in bones, and
(4) a very slowly exchangeable part of lead in bone.

The retention of lead was treated as a power function model rather than an exponential model:

$$R_t = A \cdot t^{-n}$$

where R_t = retention of lead at a specified time (t) as a fraction of the given dose
A = value of the retention at $t = 1$
n = coefficient characterizing the rate of loss from the body

Piotrowski (1971) summarized these animal studies by affirming that lead requires a particularly complex metabolic model, which has not yet been satisfactorily solved.

Mercury. Accumulation of mercury after the administration of inorganic mercuric mercury and alkyl mercury compounds follows different patterns. The rapid conversion of phenyl mercury (Gage 1964) and methoxyethyl mercury (Daniel et al. 1971) results in a distribution pattern which, after a preliminary period, resembles the distribution of inorganic mercury (Gage 1964, Ellis & Fang 1967).

Inorganic mercury—Mercuric mercury. A multicompartment model for mercury accumulation in the kidney has been used for calculations (Nordberg & Skerfving 1972) in animals. With all probability a similar type of model would be necessary if precise calculations of kidney accumulation in human beings were to be made.

The biological half-time of mercury in whole body of humans administered inorganic mercuric mercury orally has been found to be 29–41 days for 5 women and 32–60 days for 5 men (Rahola et al. 1971, Miettinen 1972). About 7% of the total administered dose was excreted according to this half-time (Rahola et al. 1971, 1973). For further data from this experiment, see section 3.2.2. Studies with experimental animals (reviewed by Nordberg & Skerfving 1972) indicate that the half-time in the kidney is somewhat longer than that in the whole body. For humans, almost no data are available on the fraction of the absorbed dose transferred to the kidney. Limited data from acute poisoning cases (Sollman & Schreiber 1936, Lomholt 1928) suggest that 10–20% of the body burden of mercury was in the kidney. However, since many of these patients died from mercury-induced kidney disease, it is likely that normal individuals would have a considerably higher proportion of the body load in the kidney. This assumption is supported by studies by Magos (1972a) in rats. When given non-toxic doses of mercury, animals with kidney damage had a smaller proportion of the body burden in the kidney than animals without such damage.

Mercury vapor. Quantitative data are not available for use in a model for mercury accumulation in the human brain after vapor exposure. The biological half-time in brain has been shown in animal experiments to be longer than in other organs (Berlin 1963, Berlin et al. 1966, Magos 1967, Berlin et al. 1969b). This is especially true for certain brain structures (Berlin 1963, Nordberg & Serenius 1969, Berlin et al. 1969b, Cassano et al. 1969, review by Nordberg & Skerfving 1972). Data from Takahata et al. (1970) and Watanabe (1971) indicate a long biological half-time, at least some years, for mercury in the brain of human beings after vapor exposure.

Methyl mercury. The data on the metabolism of methyl mercury are adequate for calculations of accumulation. Data from studies in animals and man (Berglund et al. 1971) justify the assumption that the distribution and turnover of mercury in different tissues of the body are faster than the elimination from the body as a whole. This means that a relationship exists between the concentration in each organ and the total body burden of mercury, and that a definite relationship exists among the levels of mercury in the different organs. It can also be assumed that elimination is correlated with the total body burden, i.e. that a definite fraction of this is eliminated per unit of time. If this is the case, then the course of elimination in continuous exposure can be expressed according to equation (3). Reasonably accurate calculations should be possible for the brain content at non-toxic levels.

Data on the biological half-time in brain (Åberg et al. 1969) do not support a definitely different half-time there compared to that in the body as a whole. The latter has been estimated to about 70 days on the basis of both experimental and epidemiological data on human beings (Berglund et al. 1971).

In studies by Åberg et al. (1969) the body burden in the head was measured at 10% of the total body burden. Since it is known from studies in primates other than human beings (Nordberg et al. 1971b) that the brain concentration substantially exceeds that in other tissues of the head, it can be assumed that the amount present in brain is about 90% of the total head burden.

Using the formula (3) and the above information, calculations of mercury concentration at steady state in brain have been made which show a concentration of 5 μg Hg/g brain tissue at a daily absorption of 4 μg Hg/kg body weight as methyl mercury (Berglund et al. 1971).

Other organic mercury compounds have not been investigated as extensively as methyl mercury. It is not possible at present to state any numerical values for the parameters necessary for calculations of accumulation.

III.7. METAL CONCENTRATIONS IN BIOLOGICAL MATERIAL AS INDICES OF EXPOSURE AND CONCENTRATION IN CRITICAL ORGANS

III.7.1. GENERAL ASPECTS

In assessing the risks connected with the accumulation of metals, especially those with long biological half-times, it is often desirable to use an index of accumulation, such as blood, urine or hair concentrations of the metal. When steady state is reached, there is a constant ratio between blood and tissue concentrations of the metal and, except for metabolic variations, there is also a relationship to the urinary concentration of the metal. However, for a biological material to be a good indicator of accumulation, especially for substances with long biological half-times, it is not enough that the relation should be constant at steady-state conditions it should also be reasonably constant during the period of accumulation. Blood, which transports most absorbed metals, is not necessarily a good indicator of accumulation in the critical organ. In the following subsections, data on the usefulness of blood, urine and hair values as indices of exposure or accumulation will be considered. Saliva, teeth, nails, meconium, placenta and biopsy material may also be useful material for studies of the body burden of metals.

III.7.2. SPECIFIC DATA

Cadmium. The usefulness of various biological materials as indices of exposure and retention has recently been discussed by Friberg et al. (1971) and by Nordberg (1972a).

Available evidence from animal experiments shows that the low levels of cadmium in urine during continuous exposure increase sharply with the advent of renal tubular impairment. In view of the analytical problems imposed by these low urinary cadmium levels in animals and man at the early phase prior to tubular impairment, difficulties have been met in using this as an index of body accumulation. However, Nordberg (1972a, b), by means of radioactive isotope experiments in animals, found a correlation on a group basis between body burden and urinary concentration measured before tubular proteinuria appears. On an individual basis, there was a wide scattering of data. Piscator (1972a), in a study of cadmium workers, could not find a clear relationship between cadmium concentration in urine of workers (without proteinuria) and duration of exposure (assumed to be an index of kidney accumulation). In recent studies using ^{115m}Cd on human beings, Miettinen (1972), and Rahola et al. (1973) found that the cadmium concentration in urine was correlated to body burden during two months following administration. In an epidemiological study, Tsuchiya et al. (1972a, b) estimated the biological half-time for the body compartment excreting into the urine at 13 years, not widely different from the estimated biological half-time in the liver (7 years) and kidney (17 years) in the same study. It can be concluded that urinary cadmium excretion in certain exposure situations might be useful as an indicator of accumulation of cadmium in the body provided that proteinuria is not present. However, further evidence is needed, especially concerning its relation to cadmium concentration in the critical organ.

Conclusive evidence on the relation of blood values of cadmium to the concentration in the kidneys has not been presented. Available evidence from animal experiments and human data has been compiled and discussed by Friberg et al. (1971) and by Nordberg (1972a, b). It was concluded that blood values probably do not reflect kidney accumulation of cadmium but may reflect the most recent exposure. This is also supported by data from studies in human beings reported by Piscator (1972a). He measured blood concentrations of cadmium in 20 cadmium workers who had been exposed for 1-20 years to concentrations of cadmium in air (about 0.05 mg Cd/m^3). Some showed proteinuria. There was no correlation between blood concentrations of cadmium and duration of exposure (which was assumed to be an index of kidney accumulation) in these workers.

Regarding hair values, Hammer et al. (1971) reported a correlation between the extent of exposure to cadmium and hair levels in US citizens, whereas data by Tsuchiya et al. (1971) showed random variation in hair levels among different groups of people. However, the last-mentioned study is difficult to interpret since no information on exposure was given. A correlation in animals between hair and whole body values has been reported by Nordberg & Nishiyama (1972). However, concerning experiments with human hair, Nishiyama & Nordberg (1972) showed that it was virtually impossible to remove external contamination of cadmium by various washing procedures. When there is a risk of external contamination of the hair it is evident that scalp hair values of cadmium are probably not useful for evaluation of body burden or levels in the critical organ.

Lead. The toxicologically important partition of lead between bone (passive lead) and tissue (active lead) makes it difficult to evaluate the relationship among concentrations in blood, urine and critical organs.

Westerman et al. (1965) found a rough relationship between lead in blood and lead in bone (as obtained from bone marrow biopsy specimens) for 12 lead workers. However, in biopsy samples, the ratios between cortical bone, trabecular bone and bone marrow are unknown. Such samples do not accurately reflect lead content of bone marrow, which is the critical organ.

For estimating total body burden, tissue samples, preferably the cortical bones, would be the best material. Such specimens are not easily available during life and therefore other material has been used. Turnover of lead in teeth following exposure is low and may be a good indicator for bone levels and body burden. Recent data from studies in schoolchildren show that the lead concentration in teeth of exposed schoolchildren was as much as five times greater than in those of children not so exposed (Needleman & Shapiro 1972). Because of the problem of external contamination, hair levels of lead may not be a good indicator of body burden.

One method which might be useful is the mobilization of lead from stores by EDTA. This method has been successfully used for diagnostic purposes to detect past exposures, but a quantitative relationship between differences in pre- and post-treatment blood and urine values and bone lead concentration has not been established.

In contrast to the lack of studies relating lead concentrations in blood, urine and other biological samples with those in the critical organs, there is an abundance of data relating the first two of these to current exposure (e.g. Goldsmith & Hexter 1967, Williams et al. 1969, Committee on Biological Effects of Atmospheric Pollutants 1972). Some data showing the relationship between lead in blood and current exposure have been summarized by Hernberg (1972). It was concluded that particularly lead in blood but also urinary lead reflect exposure well. In general, blood lead is more reliable than urinary lead, which fluctuates according to metabolic factors in renal handling. Since at least 90% of lead in blood is bound to the erythrocytes, a correction for hematocrit should be made in cases of anemia (Hernberg 1972).

Mercury. On the basis of experimental and epidemiological data, it has been shown that blood concentration would be a good indicator of brain accumulation of methyl mercury in chronic low exposure (Berglund et al. 1971). It was also discussed whether whole blood, plasma or blood cell values should be used as an index of exposure to methyl mercury and of retention of methyl mercury in the body and in the nervous system. It was concluded that the blood cell value was the best indicator, but that whole blood values, though less reliable, could also be used successfully. If exposure to other mercury compounds can be excluded, total mercury level in blood cells is a good index. The ratio between levels in blood cells and plasma is about 10 at methyl mercury exposure in man.

Available evidence indicates a rectilinear relation between total mercury levels in blood and hair, the hair levels being about 300 times higher than the whole blood levels for methyl mercury (Berglund et al. 1971). These conditions make hair levels a good indicator of body or brain accumulation of mercury. Since hair from different sites grows at different rates and since hair may take up some metals externally, great care must be taken when sampling hair to provide (1) freedom from external contamination and (2) standard type and length of the hair sample. Possible external contamination should be remembered, especially with regard to industrial exposure, since it is likely to involve airborne mercury.

Urinary excretion of methyl mercury is correlated to blood levels. Because of the low levels of methyl mercury in urine and the possible presence of inorganic mercury, total mercury in urine is not a reliable measure of body burden of methyl mercury. For organomercury compounds other than the alkyl group, it is probable that both urinary excretion data and blood values give some information about recent exposure (Nordberg & Skerfving 1972).

For mercuric mercury, there is not a constant ratio between mercury concentrations in blood and critical organs according to animal experiments (Berlin 1963, Nordberg & Skerfving 1972). Studies in man by Miettinen (1972), following a single oral dose, showed that the biological half-time in blood (20 days) is half of that in whole body (40 days).

It seems probable that blood is not a good index for evaluation of mercury accumulation in kidney.

Animal experiments and epidemiological data do not support urinary mercury as a good index of kidney concentration (Friberg & Nordberg 1972).

With regard to mercury vapor, a correlation on a group basis between blood values and exposure has been reported (Goldwater 1964, Smith et al. 1970). In individual cases urinary values may give conflicting conclusions about the risks for poisoning, whereas in larger groups of people urinary mercury levels might serve to some extent as an indication of exposure (Smith et al. 1970).

III.8. GENERAL DISCUSSION AND NEED FOR FURTHER RESEARCH

The purpose of the meeting was to examine the available information on models for the absorption, excretion and accumulation in critical organs of toxic metals with special emphasis on general principles. Although such models were available for some parameters, the differences were evident among the three metals especially examined, cadmium, lead and mercury. Furthermore, although data exist, it is obvious that there are gaps in what is known of the behaviour of metals both during and following absorption within the organism. Some of these gaps are such that a critical evaluation of risks related to different exposure situations is difficult.

The terms to describe the processes leading to accumulation were scrutinized. The concept of critical concentration in a particularly susceptible organ or tissue was defined as the earliest stage at which functional damage exists there. Models of accumulation were attempted, based on the biological half-time of the metal in the critical organ, but these were limited by lack of data on whether a single or a number of exponential functions would provide the better description or, indeed, whether an exponential function would be appropriate at all. More complicated multicompartmental models, in fact, might better describe the processes involved. However, the simple exponential equation and the concept of biological half-time implicit to it were found, in some cases, to be useful in interpreting experimental data, as for example in the case of methyl mercury.

With regard to absorption by inhalation, there are fairly good data on average deposition of different particle sizes. The gap here concerns the limited understanding of the wide fluctuations among individuals. Furthermore, the influences of age, physical activity, particle concentrations in inspired air, and respiratory disease require further definition. Internal redistribution of particles deposited in the alveoli is poorly understood with respect to the dose of toxic materials to specific structures in this part of the lung.

It is not known to what extent metal particles, once they are deposited in the alveoli, are subject to transpulmonary absorption or to translocation via the mucociliary escalator. Data on total alveolar clearance are extremely scarce, particularly with regard to particle size and chemical form of the metal. For example, differences among very soluble metal chlorides, moderably soluble metal oxides and relatively insoluble metal sulfides are not sufficiently understood. Interindividual health and constitutional factors have been shown to be significant for clearance rates and should be better quantified.

Absorption following ingestion and the excretion of metals are important functions of the gastrointestinal tract. However, fewer data are available on the basic processes involved than is the case with absorption by inhalation. Most current experimental work concerns fecal elimination of metal or net gastrointestinal excretion. The mechanisms of absorption and excretion are not fully understood, nor have kinetic interrelationships been quantitatively determined. Although some balance studies in humans have been reported, more data are needed to quantify total gastrointestinal absorption in relation to variation with age, diet and disease states. Especially urgent are investigations into physiological differences between children and adults that appear to be of significance to health as far as human exposure to metals is concerned.

Experimental studies in animals are needed to determine the direct and interrelated roles of various factors influencing gastrointestinal absorption. Such studies should differentiate total from net excretion as may result from pancreatic juice, bile, secretions from intestinal glands and the shedding of epithelial cells into the intestinal lumen. Models for gastrointestinal absorption and excretion valid for humans can be designed only by the combined use of human balance studies and experimental animal data.

With regard to the transport of these metals within the body and their distribution to organs and tissues, the important role of the small, diffusible plasma fraction for the exchange of metals between the larger fraction bound to plasma protein and among the vascular, interstitial and intracellular compartments was stressed. Metals are distributed non-uniformly and preferential organ accumulation occurs in a pattern that is specific for a given metal, but may be different for its organic compounds. However, the metal may be released from its initial binding sites to be taken up by organs with more stable metal binding. Basic research on the nature of metal binding by various compounds of body fluids in man and experimental animals is needed to identify factors responsible for transport of metals within the body. Physiological and pathological conditions that may have an effect on the uptake, binding and release of metals by tissues and organs should be studied to enable better understanding of relations between concentrations in body fluids and in critical organs.

Potentially toxic metals exhibit marked differences among them with regard to binding to and passage through the human placental barrier. There is a need for more detailed studies on metal levels in cord blood, tissues from infants, and placental tissues from mothers. Experimental studies involving primates should complement the human studies so that a broad range of metal doses related to gestational stages can be evaluated.

Although the brain is a critical organ for metals such as lead and mercury, little is known about mechanisms involved in the uptake and turnover of metals in nervous tissue. Available data indicate a complex interrelationship between metal turnover and the nervous system compared to other organs of the body. Animal studies are required to elucidate the basic mechanisms involved.

With regard to excretion, understanding of the mechanisms involved in the renal excretion of metals should be improved. The complex process of urine formation, together with the high variability among individuals in many cases prevents more efficient use of urinary levels for evaluation of body burdens of metals. The identification of nutritional and other physiological factors which may influence the renal handling of metals may help interpretation of metal concentrations in urine. Metals in milk constitute both an excretory and an exposure route and mammary excretion processes in man and domestic animals need to be defined and quantified.

Indices of metal accumulation and exposure are urgently needed. It has been shown that metal levels in available biological material (blood, urine, hair, etc.) might give an approximate indication on recent exposure but with few exceptions cannot be used to calculate metal concentration in critical organs or in whole body. Consequently, in many cases, it is impossible to tell on the basis of sample analysis whether accumulation in the critical organ is progressing and how far metal concentration in the critical organ is from the critical level. The reason for this lack of knowledge is twofold. One is the proper numerical values which could be set up in an equation; the other is the lack of a proper mathematical model which is adequate to describe with approximate accuracy the complexity of metal accumulation in the body.

Available data for metal retention in organs are mainly derived from experimental animals or from adult humans in the medium age range. There is a need to obtain additional data from both young and old normal individuals.

It would be valuable to establish the total exposure for defined populations so that changes in tissue levels can be related to changes in the environment. In this connection it would be of value to organize systematic collection of data on metal concentrations in critical organs from subjects with known exposure, together with indices of exposure such as blood, urine, hair, teeth and placental tissue. Storage of samples in tissue banks will facilitate the evaluation of time trends in relation to accumulation and concentration. Further estimates may be performed with improved and new analytical methods. In order to make such data collection useful, collaboration among institutes for the standardization of analytical and other relevant procedures on an international level is of paramount importance.

There is a lack of epidemiological data on the parameters discussed above in populations with industrial exposure. This lack is even more striking in relation to the growing number of such studies on populations with general environmental exposure, which is usually at a much lower level. There is a need in industrially exposed populations for standardized, wherever possible collaborative, epidemiological studies, where cohorts can be followed in time and where groups can be related to each other. With some occupational exposures to the less common metals, only small groups may be available for study in any one country, so that international collaboration in epidemiological studies would again be of value.

III.9. ACKNOWLEDGMENTS AND LIST OF WORKING PAPERS

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During the meeting in Buenos Aires the task group received assistance from Dr. N. Cozzi; Mrs. Kersti Dukes supervised the secretarial work. Editorial assistance for this report was given by Pamela Boston.

(*Referred to at the present as Author*, 1972. *All of the working papers listed below will be published in the Proceedings of the XVIIth International Congress on Occupational Health*).

Roy E. Albert (in collaboration with M. Lippmann, D. Bohning & N. Nelson): Gastrointestinal translocation of inhaled particles.

Maths Berlin: Transport, distribution and retention of toxic metals in animals and man with special reference to inorganic compounds of mercury and lead.

John F. Finklea (in collaboration with J. C. Creason, V. Hasselblad, D. I. Hammer, L. F. Priester, S. H. Sandifer & J. F. Keil): Transplacental transfer of toxic metals.

Lars Friberg (in collaboration with Tord Kjellstrom): Interpretation of empirically documented body burdens by age of metals with long biological half-lives with special reference to past changes in exposure.

Robert A. Goyer: Cellular and subcellular effects of lead.

Richard Henderson (in collaboration with H. P. Shotwell & L. A. Krause): Significance of total, ionic, and elemental mercury in urine for establishing biological threshold limit values.

Sven Hernberg: The value of lead analyses in blood and urine as indices of body burden, accumulation in critical organs and exposure.

Albert C. Kolbye, Jr. (in collaboration with R. E. Shapiro & S. I. Shibko): Analysis of parameters affecting regulation of heavy metals in foods in the United States.

Laszlo Magos: Information on accumulation of metals in critical organs in relation to the chemical structure of the substance.

Jorma K. Miettinen: Gastrointestinal absorption and whole-body retention of toxic heavy metal compounds (methyl mercury, ionic mercury, cadmium) in man.

Paul E. Morrow: Pulmonary absorption of toxic metals.

Gunnar Nordberg: Models used for calculation of accumulation of toxic metals.

Tor Norseth: Gastrointestinal absorption and excretion of toxic metals in animals and man.

Emil A. Pfitzer: Absorption, distribution and retention of toxic metals with special reference to inorganic and organic compounds of lead.

Magnus Piscator: Excretion of metals in relation to functional impairment of organs (referred to as 1972b).

Samuel Shibko: See Albert Kolbye, Jr. above.

Ana Singerman: Biochemical background for functional impairment induced by toxic metals.

Kenzaburo Tsuchiya (in collaboration with M. Sugita & Y. Seki): A mathematical approach to deriving biological half-time of cadmium in some organs calculation from observed accumulation of the metal in organs (referred to as 1972b).

Jaroslav Vostal: General mechanisms of renal and biliary excretion of toxic metals.

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SECTION IV

EFFECTS AND DOSE-RESPONSE RELATIONSHIPS OF TOXIC METALS

(To be published in conjunction with the working papers from the meeting by Elsevier Scientific Publishing Company.)

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Editor's Preface
to
Effects and Dose-Response Relationships of Toxic Metals[*]

The field of environmental toxicology of metals has attracted considerable interest in the general and scientific communities during recent years. Although it is well recognized that health effects of metals in the general environment in some instances have been demonstrated to be closely related to events in the overall ecosystem, the present volume is focused on human toxicology, always an important part of the total evaluation of environmental toxicology.

The book constitutes the proceedings from a meeting of thirty-six experts on metal toxicology and epidemiology from twelve countries, which was organized by the Subcommittee on the Toxicology of Metals under the Permanent Commission and International Association of Occupational Health and held in Tokyo, November 18-23, 1974. The first part of the volume (Chapters 1-7) is the consensus report, worked out by the participants and circulated among them prior to the meeting. Contributions of original reports of experimental and epidemiological data, as well as reviews on various topics within metal toxicology, made up the working papers which are presented in the other parts of this book (Parts A1-C4). While it is evident that the contributions vary considerably in length and style of presentation, and a certain overlap sometimes occurs, the Editorial Committee has considered it valuable to include most of the contributions in the present volume so as to give the reader a comprehensive review of what written information was available for the discussions in Tokyo. This does not imply that the Editorial Committee and its reviewers have always completely agreed on the scientific approach and interpretation of data by individual authors. The working papers (referred to in the table of contents as contributed articles), though they appear after the consensus report in this volume, were written prior to the meeting, meaning that the terminology and concepts defined in the report have not been consistently used in them.

Further details concerning how the meeting was organized are given in the introduction to the consensus report. The final version of the report and the book were reviewed by the Editorial Committee during a meeting in North Carolina, U.S.A., in March 1975.

It is believed that this volume, and the consensus report in particular, constitutes a unique document in defining concepts in metal toxicology. It also provides an up-to-date review of the toxicology of cadmium, lead, mercury, and their compounds, as well as an insight into interactions among them and with other metals. This volume thus represents a considerable total effort on the part of all of the participants. The Editor and the Editorial Committee wish to thank each contributor for his work and cooperation in completing the document.

On behalf of the Editorial Committee,

Gunnar Nordberg

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SECTION IV

EFFECTS AND DOSE RESPONSE RELATIONSHIPS OF TOXIC METALS

IV.1 BACKGROUND

The Subcommittee on the Toxicology of Metals under the Permanent Commission and International Association on Occupational Health, at meetings in Slanchev Bryag, Bulgaria, 1971, and in Buenos Aires, 1972, discussed questions on accumulation of toxic metals with special reference to their absorption, excretion and biological half-times. As a result of the meetings, two reports have been published (Dukes and Friberg, 1971, and Task Group on Metal Accumulation, 1973). At the last mentioned meeting it was decided to continue the work on metal toxicology by arranging a third meeting where questions concerning effects and dose-response relationships of toxic metals would be discussed in particular. As a result of this decision a working group, the participants of which were invited by the subcommittee, convened in Tokyo, November 18-23, 1974. In addition to the participants about 30 observers attended the plenary sessions and occasionally took part in small group discussions. The participants are listed separately. The meeting, which took place at the Research Institute of Industrial Safety, Tokyo, was officially opened in welcoming speeches by Dr. Yoshio Ohtaki, Executive Director of the Japan Industrial Safety Association and also Secretary General of the meeting; Mr. Kinnosuke Tohmura, Director, Department of Labor Standards of the Ministry of Labor; Dr. Velimir Vouk representing the World Health Organization, Geneva; and Dr. Juko Kubota, Director, Japan Industrial Health Association and Honorary Chairman of the meeting. Opening remarks were given by Dr. Lars Friberg, Chairman of the Subcommittee.

Dr. Lars Friberg was elected Chairman of the meeting, Dr. Kenzaburo Tsuchiya was elected Vice-Chairman, and Dr. Gunnar Nordberg, Secretary of the Subcommittee, was elected Rapporteur.

The participants of the meeting had prepared working papers dealing with specific topics in the area of effects and dose-response relationships and these were circulated in advance to all participants. Based on the working papers, Dr. Gunnar Nordberg had prepared a draft report which was sent to participants and observers in advance. The discussions at the meeting were centered on this draft report, which was revised during the meeting. The entire report was read, discussed, and approved by the group of participants as a whole. The detailed preparatory work was performed in five subgroups: one general group (Chairman: Dr. Vouk), one group on cadmium (Chairman: Dr. Vostal), one on lead (Chairman: Dr. Goyer), one on mercury (Chairman: Dr. Suzuki), and one on interactions (Chairman: Dr. Parizek). Final editing of this report was done by the Rapporteur (Dr. Nordberg), with assistance of an editorial committee consisting of Drs. Friberg, Horton, Pfitzer, Tsuchiya, Vostal and Vouk. Administrative editorial assistance was provided by Ms. Pamela Boston.

As was the case during the two previous meetings arranged by the Subcommittee it was decided to take into consideration not only experience from occupational exposure, but also that from exposure through other media, including ambient air, water, and food. One reason for this was that effects would be related to the total exposure from the environment. Further, much evidence from experience related to the general environment was available for certain evaluations concerning relationships between exposure to metals and effects.

The term "toxic metals" is used in this report to include metallic elements. It also sometimes includes nonmetallic elements, which, under certain conditions, may exhibit properties of metals to some degree, e.g. selenium. In a strictly scientific sense, all metals, in fact all chemicals, are toxic when too much reaches vulnerable sites in the body. This report centers on metals whose toxic potentials have been recognized as serious problems for humans. Thus, primary emphasis has been given to lead, mercury, and cadmium, but a few other elements are also discussed. The term "metals" may occasionally be used broadly to include metals in elemental form, metals as inorganic or organic salts, or organometallic compounds. Detailed data in the report will include, when possible, the description of the specific form of the metal or metal compound.

Because the present meeting to a large extent is a continuation of the meeting held in Buenos Aires, 1972, dealing with "Accumulation of toxic metals with special reference to their absorption, excretion and biological half-times", the conclusions reached there have been taken advantage of here. Reference is therefore made repeatedly in this paper to the published report from that earlier meeting (i.e. TGMA, 1973).

A summary of the most important conclusions from the earlier meeting will be incorporated here for easy reference, since this information constitutes a substantial part of the considerations on the "dose" in the dose-response relationships which were discussed at the present meeting. The main focus of this meeting has been on the effects of certain doses of metals as well as associations between such doses and effects.

The earlier meeting outlined the general philosophy behind metabolic models and critical organ concentrations for evaluation of metal toxicology (TGMA, 1973). This philosophy is based on the concept of a threshold dose for the occurrence of adverse effects in the organ first displaying such effects during the exposure to toxic metals. The basis for a threshold dose concept lies in the interference of metals with certain biochemical processes and structural components, e.g. membranes, organelles and the enzyme systems within the cells of the body. Usually a certain reserve capacity on the enzymatic level allows the cell to absorb certain amounts of metal without undergoing evident functional changes. By the use of the critical-organ-concentration --metabolic model approach, certain features of dose-effect relationships for toxic metals may be explained, for example effects resulting from accumulation due to long-term exposure to substances with long biological half-times. It is felt that the approach is helpful in many cases to interpret and to establish dose-response relationships.

The group was aware that much important work was under way on an international level under the auspices of other international organizations; particular reference was made to the intensive project of the World Health Organization environmental health criteria documents, which even now, but more so in the future, will be of great merit for the endeavors within the Subcommittee on the Toxicology of Metals, whose work could be of mutual importance for the work within WHO. It was therefore clear that a close collaboration, as was the case during this meeting, should be established between the Subcommittee and the World Health Organization. Also, the work within other organizations, as, for example the International Union for Pure and Applied Chemistry (IUPAC), and the International Council of Scientific Unions (ICSU) and its Scientific Committee on the Problems of the Environment (SCOPE) was referred to as being of great relevance for the work within the Subcommittee.

IV. 2 CONCEPTUAL CONSIDERATIONS: CRITICAL ORGAN, CRITICAL CONCENTRATION IN CELLS AND ORGANS, CRITICAL EFFECT, SUBCRITICAL EFFECT, DOSE-EFFECT AND DOSE-RESPONSE RELATIONSHIPS

The definitions to be given in this section of the report were much discussed during the meeting, and it was pointed out that the resulting concepts might clarify some problems involved in the early identification and prevention of adverse effects of exposure to toxic metals. Since the prevention of adverse health effects is the primary objective of environmental health, it was hoped that the concepts defined here and used in the other chapters of the report could be considered by other workers in the field of toxicology and environmental health.

The *critical concentration for a cell* was defined by the Task Group on Metal Accumulation, 1973, as the concentration at which undesirable functional changes, reversible or irreversible, occur in the cell. The *lethal concentration for a cell* was defined as the cellular concentration sufficient to cause death of the cell.

The present meeting suggested that "adverse" might be a more appropriate term than "undesirable". It also stressed that critical concentration should always be related to a defined effect.

Critical organ concentration was defined as the mean concentration in the organ at the time any of its cells reaches critical concentration (TGMA, 1973). This meeting noted that in most practical cases a certain number of the most sensitive type of cells in the organ will be affected in order for the critical organ concentration to be reached. The critical organ concentration may be considerably higher or lower than the critical concentration for a particular cell. This is possible since the type of cell that first attains the critical concentration is not necessarily the type of cell with the highest concentration. The usefulness of the concept "critical organ concentration" stems from the lesser likelihood, generally, of this concentration's displaying fluctuation in relation to the appearance of effects, compared to the whole body concentration or other "dose" or exposure estimates.

Since there exists biological variation in sensitivity among individuals, a certain interindividual variation is to be expected also in critical organ concentration. The critical organ concentration may even be subject to variation both in an individual and in a population. This has recently been pointed out for this meeting by Tsuchiya et al. (1975) and Sakabe et al. (1975) for cadmium and by Suzuki and Shishido (1975) for methylmercury compounds. Further detailed considerations for the various metal compounds will be given in Chapters 3-6.

Critical organ[x] is defined as that particular organ which first attains the critical concentration of a metal under specified circumstances of exposure and for a given population.

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[x] The definition of the term "critical organ" is consistent with earlier definitions given in this report (and TGMA, 1973), but differs from the definition given by, e.g., the International Commission on Radiological Protection: "Critical organ is defined as the organ of the body whose damage by radiation results in the greatest injury to the individual (or his descendants). The injury may result from inherent radiosensitivity or indispensability of the organ, or from high dose, or from a combination of all three." (ICRP, 1959). A different approach has been chosen here because it has been considered advantageous from the point of view of preventive medicine. The present definition implies that if critical effects in critical organs are identified and prevented, no adverse effects for the whole organism shall appear. Certainly effects in organs which might be "critical" from the clinical point of view (i.e. signalling a grave danger for the patient) would be prevented. Other definitions of "critical organ" relating the term to clinical disease or damage to the individual as a whole, may not necessarily achieve the goal of preventing all types of adverse effects. Under certain circumstances the term "organ" may not be adequate and one could talk about critical system or critical tissue. In this report we have not striven after such exact usage in this case and the term organ is used broadly.

The organ (system, tissue) of greatest accumulation is not necessarily the critical organ, e.g., in lead exposure the highest concentrations may be reached in bone without any identifiable effect; the bone is therefore not the critical organ. Sensitivity of organs may show interindividual variability due to metabolic and other factors that in some cases may make one organ critical for one person and another organ critical for another person. It is also evident that the organ that becomes critical may vary with the type or route of exposure (single, repeated, short- or long-term exposure; oral, inhalation, etc.).

For these reasons several organs may be classified as critical for a defined metal or metal compound. For example, for short-term exposure to high concentrations of cadmium in air, the lung may be the critical organ, whereas for long-term exposure to lower concentrations by the same route, the kidney may be the critical organ.

The organ that becomes critical may also vary depending on the characteristics of the population exposed. There is some evidence, for example, that in children exposed to lead, the brain may be the critical organ, whereas this is not necessarily the case for adults.

The above given definition of critical concentration in the critical organ represents a defined point in the relationships between dose and effects in the individual, namely the point at which an adverse effect is present. This is the critical effect. This critical effect may or may not be of immediate importance for the health of the whole organism. It is of interest to discuss doses higher than those corresponding to the critical concentration, and particularly to discuss the relationship between adverse effects at the cellular level and significant hazards to the health of the organism.

At an exposure level that is lower than the one giving critical concentration of metal in the critical organ, some effects might occur that do not impair cellular function, but still are evident by means of biochemical or other tests. These effects are defined as subcritical effects. The concentrations producing such effects are defined as subcritical concentrations, and each must be related to a defined effect. The biological meaning of a subcritical effect is sometimes not known; in some cases it only indicates that an exposure has taken place; in some cases it may be a sign of an adaptation; in other cases it may be a precursor of a critical effect. The last mentioned type of subcritical effect may be especially useful in preventive medicine, e.g., when it is possible by a biochemical test to determine a graded subcritical effect that precedes the development of the critical effect. For example, in lead exposure, an inhibition of the enzyme ALA-dehydrase in the cells of the bone marrow is a subcritical effect which precedes an increased level of ALA in blood and urine and the occurrence of anemia (critical effects). A decrease in ALA-dehydrase activity in blood is an example of an indicator of a subcritical effect of lead exposure. A general discussion on types of effects that should be taken into consideration when defining critical organ concentration and the possibilities for their detection will be elaborated upon in Section IV. 4 of this report.

The term "effect" is used to mean a biological change caused by an exposure. Sometimes this effect can be measured on a graded scale of severity, although at other times one may only be able to describe a qualitative effect that occurs within some range of exposure levels. When data are available for the graded effect, it is apparent that one may establish a relationship between dose (usually an estimate of dose) and the gradation of the effect in the population; this is the dose-effect relationship. An example may be the relationship between lead concentration in industrial air and the concentration of ALA in urine samples from workers.

Ideally a dose-effect relationship is established with measurements on many individuals over a range from minimum to maximum effect. The curvilinear relationship that represents the best fit to all of the data points should be expressed in terms of mean values and their standard deviations at various doses. In other words the scatter of data will usually lead to confidence limits around the mean values, and these expressions of degree of uncertainty should be evaluated wherever possible.

The term "response" is used to mean the proportion of a population that demonstrates a specific effect, and its correlation with estimations of dose provides the dose-response relationship. For example, a dose-response relationship might compare different lead concentrations in industrial air (estimates of dose) with the percent of the exposed workers that have greater than 5 mg ALA/liter of urine.

At times one measures a change (in terms of frequency or degree) that is not a biological change, e.g., lead in the blood or cadmium in the kidney. When such data are correlated graphically with the degree of exposure, they may have the same form as a dose-effect or dose-response relationship; however, such relationships simply define the correlation between two different estimates of dose, according to the principles and purposes of this report.

To establish dose-response and dose-effect relationships requires much data that are often lacking for human beings with regard to many metals. In such cases, in which only limited data are available, knowledge of general principles governing the action of metals may sometimes permit approximations of such relationships.

In addition to these definitions of the words response and effect, which will be further elaborated upon in section IV.5.1, the term dose has been given a specific meaning and usage (section IV.3.1.1).

IV. 3. DOSE

IV.3.1 General considerations on dose and exposure; possibilities for their quantitation

Occupational exposure to toxic metals or their compounds occurs mainly through inhalation of dusts or vapors, although ingestion and skin absorption may also play a substantial role. The general population may be exposed via air, water, and food. Smoking may also contribute to the intake of some metals. The total exposure of the occupationally exposed population may include a significant contribution from food and water in the general environment as well. Hence, the relationship of these two routes of exposure to other routes must be considered.

Difficulties may arise if all of the different routes of exposure are not taken into account. In general environment studies, for instance, it may not be useful to correlate exposure from airborne lead with blood lead levels if the exposure of the population to lead via food is not simultaneously measured (review: Piscator, 1975). When dealing with the exposure to metals via food, the chemical form of the metal is a primary consideration. In many instances, merely analyzing for the metal as an element is not adequate. There is little information on the forms of metals in food, with the exception of mercury in fish. Mercury in food may be in the form of methylmercury and/or inorganic mercury. Lead in plants may exist as lead phosphate and may also be complexed with organic acids. In soft tissues of animals, complexes are formed with proteins and/or lipoproteins. Cadmium may be found complexed with low-molecular-weight proteins, as in metallothionein, and also with other types of proteins.

A source of error when dose estimates are to be made concerning exposure via food is the fact that the levels of metals in food may be unpredictable and not easily controllable (review: Shibko et al., 1975). The vastly different dietary habits of exposed individuals lead to differences not only in exposure but also in interactions between dietary components, in turn possibly leading to differences in metabolism and effects.

A quantitative assessment of the dose or even of accumulated exposure is especially difficult as regards long-term exposure. Assessment of dose by analysis of biological samples, blood for example, may be possible, but may be burdened with several difficulties. The following sections of this report will put special emphasis on discussion of possibilities and difficulties involved in using metal determination in biological materials as dose estimates.

IV.3.1.1 Definition of "dose"; general considerations on its assessment

Ideally, the dose should be defined as the amount or concentration of a given chemical at the site of effect, i.e., where its presence leads to a given effect.[x] Unfortunately, the determination of this amount is often not possible in practice. It is therefore clear that the dose may have to be estimated in various ways, some common ones of which are -

a) From experimental exposure: injection; ingestion, including feeding experiments; inhalation; and dermal or other topical applications. In all these cases the dose is estimated from the amount or concentration applied, the time and, when available, relevant deposition, retention, and absorption factors.

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[x] This definition of the concept "dose" was considered during the present meeting to be useful for establishing dose-response and dose-effect relationships at various structural levels. However, there was no objection to the term "dose" in its classical sense, meaning amount administered to, e.g., an experimental animal. When the term "dose" is used in this classical sense in this report it will always be specified as "administered dose", "oral dose", "injected dose", etc.

b) From occupational exposure: This usually involves the concentration in air, rate of inhalation, time, and the appropriate deposition, retention and absorption factors. In addition, dermal exposure and ingestion during work time should be considered, noting that the worker is also exposed to ambient air, drinking water, and food, as a member of the general population.

c) From general environmental exposure: This involves inhalation of air, ingestion of food and water, and exposure from other sources including drugs, consumer products, tobacco smoke, pica in children, various beverages, etc. The same considerations as to amount or concentration, time, and deposition, retention, and absorption factors apply.

d) From measurement in bodily compartments: This involves indicator (or index) media such as blood, urine, feces, sweat or hair. In addition, compartments such as organs, tissues, specific groups of cells, and subcellular components may be useful. This is discussed in detail in subsequent sections of this chapter.

The accuracy and precision of the various estimates of dose will depend upon the validity of sampling and analytical methodology. The estimates must also consider the interactions and biotransformations of the chemical in the organism (see sections IV. 3.3 and IV. 3.4.

It is evident from this discussion that the term dose as used in this report will necessarily be an estimate of the dose as defined at the beginning of this section. Therefore, whenever the term dose is used it should be made as clear as possible how the dose was estimated, including the specific units, the physical form and the chemical species involved.

IV.3.1.2 Quantitative estimations of dose

IV.3.1.2.1 General Aspects

In the practical situation, when trying to establish the extent of health hazards in industry or in the general environment, chemical analysis is necessary of metal concentrations in air, drinking water, food products or indicator (or index) media, like blood, urine, and hair. The problems related to such analyses are often considerable and are partly due to the difficulties in performing accurate chemical determinations of metal concentrations in the various biological materials mentioned. However, problems related to sampling may be of equal or greater weight, particularly in relation to the variations in concentrations of the materials sampled. In addition, variations in consumption of food, inhalation of air, the nature of the population studied, and uncertainties in relationships between concentrations in the indicator (or index) media and the organs will contribute to the overall errors. Further, as regards metals with long biological half-times in the critical organ, estimations of the history of exposure are necessary in order to calculate the dose from analyses of environmental samples.

The difficulties can be exemplified as follows. Blood concentration is sometimes taken as a measure of the dose in spite of the absence of adequate knowledge as to the relationship between the blood concentration and the exposure, or the blood concentration and the concentration in the critical organs. When, in such cases, no correlation is found between the blood concentration and a certain effect, it can be assumed that the blood concentration is not a good indicator (or index) of the concentration in the critical organ, rendering the approach useless. Data and discussions given elsewhere (TGMA, 1973), which can serve to illustrate the

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usefulness of metal concentrations in indicator (or index) media for the estimation of the dose and their relation to the concentration in the critical organ, will be summarized in section IV.3.4.

IV.3.1.2.2 Chemical analysis of metal concentrations in food, air, water, and various biological indicator (or index) media

Problems related to analysis of metals in biological materials are substantial. In addition to the problems in connection with elemental analysis, there are those with identification of the chemical species and its physicochemical properties, e.g., organomercury compounds. It is not the intention of the present report to give a detailed discussion of the analytical chemistry of metals, but the following comments are meant to give an appropriate point of departure for later topics.

The main problem in the determination of metals in industrial air is generally not the final chemical analysis, but the sampling technique, which might be inadequate. The sampling site may be a distance away from the actual place of exposure, resulting in data that do not reflect the true exposure. Whenever possible, personal sampling equipment should be distributed. This may be feasible in occupational exposure studies, but very difficult in epidemiological studies involving general population groups. With regard to analysis of drinking water, metal concentrations are often low and may necessitate concentration of the sample, which may introduce errors. For other media such as blood, urine, and certain food products, concentrations of metals are often low and interfering substances may be present. Analytical errors thus may become serious stumbling blocks (for further discussion see Piscator, 1975).

Blood lead concentrations are often not accurately determined in spite of the relatively long experience with such analyses. In a recent interlaboratory comparison among 66 European laboratories employing varying analytical techniques, differences with a factor up to, and sometimes more than, 10 were noted in the results (Berlin, A. et al., 1974). Similar examples are available from other studies on metals and media. This reveals the need for inter- and intralaboratory quality control programs on a more systematic basis.

In conclusion, it should be pointed out that a critical approach toward sampling and evaluative techniques is a prerequisite for assessing published results. Even a theoretically appropriate, precise, and accurate method will not automatically ensure reliable results. Obviously, reliable dose-response relationships cannot be obtained from erroneous exposure data.

IV.3.2 The metabolic model and its relation to metal concentrations in biological media as indicators (indices) of exposure and of concentrations in critical organs

A metabolic model describes,in qualitative and quantitative terms, the processes (i.e., type and rate) of absorption, distribution, deposition, biotransformation[x], retention, accumulation, and excretion. It also includes other chemical and physical interactions (e.g., protein binding) that a metal or its compounds may undergo in their passage through the organism. Other terms have been used to describe such relationships, the most common being "pharmacokinetics". The present meeting has decided upon the term "metabolic model".

The metabolic model includes data concerning absorption of metals after inhalation, ingestion, or contact with the skin, as well as transplacental transfer of metals. The relationship between metal concentrations in biological materials, as indicators (or indices) of exposure, and concentration in critical organs is of special interest.

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[x] The term "biotransformation" when applied to metals will be understood to refer to changes in the oxidation state of the metal and the formation and cleavage of covalent organometallic bonds.

The various components of the metabolic model have been discussed in detail and specific data on lead, mercury, and cadmium and their compounds given by TGMA (1973). The following summary provides the most important data brought forth and conclusions reached at that time. In addition, more recent data have been included as a result of discussions during the present meeting.

It is obvious that it would be extremely valuable if precise data on the various parameters included in the metabolic model for a certain substance could be deduced from theoretical considerations based on generally valid principles governing these parameters. Unfortunately, it became equally as obvious from discussions at the Buenos Aires meeting that such generally valid principles could not be put forth to any notable extent, except for certain pulmonary absorption parameters. It was thus evident that specific data concerning absorption, distribution, and excretion for every individual metal and metal compound must be determined experimentally, in order to construct a metabolic model that would be sufficiently precise for practical purposes. Such data can be obtained in various ways, e.g. from studies of substances labelled with radioactive or stable isotopes and administered by the various exposure routes, to experimental animals, and even to human beings.

IV.3.2.1 Absorption

IV.3.2.1.1 Absorption by inhalation

Though usually of a more obvious nature for human exposures in the industrial environment, pulmonary absorption of metals may also be of importance in the general environment, including exposures from tobacco smoking. Metals may be taken into the respiratory system in the form of particles, gases or vapors. General principles of the absorption of metals in these various airborne forms were presented by TGMA (1973). With regard to absorption of metals and metal compounds in particle form, it was concluded that absorption was influenced by three processes in the lung, namely: deposition, mucociliary clearance, and alveolar clearance. For an explanatory model of these different processes, the model by the Task Group on Lung Dynamics (1966) was used. After deposition in the nasopharyngeal, tracheobronchial or alveolar (pulmonary) compartments, the metal may be transported by mucociliary action to the gastrointestinal tract or absorbed into the blood. The fraction deposited in the nonalveolar compartments of the lung participates mainly in the first-named type of translocation. The portion deposited in the alveolar compartment is more likely to be absorbed but may take different routes.

Deposition of particles in alveolar and tracheobronchial-nasopharyngeal compartments of the lung was considered to be dependent on the physical characteristics of the aerosol (such as the size and density of the particles) and also on the parameters of respiration (nose or mouth breathing, tidal volume and respiratory rate). The anatomical variability of the lung may have major relevance for deposition. Variation depending on such interindividual variability may amount to a factor of 2-to-3. The portion of the total deposition that falls within the tracheobronchial and nasal compartments is generally not absorbed into the body unless the aerosol particles consist of easily soluble salts. This proportion of the metal is translocated into the gastrointestinal tract.

The proportion deposited in the alveolar compartment will be cleared from this compartment by (1) transport onto the mucociliary escalator and translocation into the gastrointestinal tract, (2) deposition for a long time in the pulmonary tissue or (3) passage through the pulmonary tissues into lymph and blood. As concerns particles with low solubility, it is not known either in human beings or in animals, how large a part of the alveolar clearance results from transfer of particles to the mucociliary escalator and how large a part from their absorption through the lung. The proportion absorbed was, however, estimated to range between 50 and 100% of the material deposited in the alveoli. Taking this variation into consideration, the total bodily absorption of particles with different sizes between 0.1 and 10 µm MMAD and with a gastro-intestinal absorption of 5%, was calculated to range from about 5 to 50% of the inhaled amount (TGMA, 1973).

Deposition of gases or vapors of metals in the respiratory tract depends on water solubility. Highly water-soluble gases are dissolved in the mucous membranes of the nasopharyngeal and tracheobronchial systems and never reach the alveoli. Less soluble gases, on the other hand, do reach the alveoli. Depending on their ability to penetrate the alveolar structure, they are absorbed to varying degrees. An example of a metal which occurs in vapor form is mercury. Its vapor reaches the alveoli and easily penetrates the alveolar membrane, resulting in a high extent of absorption (about 80%).

Insufficient data are at hand concerning what influence age and pathological conditions would have upon the pulmonary absorption of metals. It is known that such conditions as chronic bronchitis and cigarette smoking may affect bronchial deposition and mucociliary clearance and thus also pulmonary absorption.

IV.3.2.1.2 Absorption by ingestion

Absorption of metal compounds after ingestion via food and beverages is a major source of exposure, but a substantial part of metals in particle form deposited in the lung may be transferred to the gastrointestinal tract by mucociliary clearance. Gastrointestinal absorption of metals has been discussed by TGMA (1973) and has also been subject to some discussion during the present meeting (reviews: Goyer and Rhyne, 1973; Shibko et al., 1975), especially with regard to the recently obtained data on influence of age and the interactions of various essential as well as non-nutritive constituents of food in relation to the absorption of toxic metals.

The vastly different absorption rate between organic and inorganic compounds of mercury is well known (TGMA, 1973), but reasons also exist to believe that there could be differences in absorption depending on the type of inorganic compound occurring in food as well as differences depending on other components in the diet.

Studies with experimental animals and man have shown that other substances in the diet, such as dietary fiber, phytate, protein, and lactose, may have significant effects on the absorption of metals. Reviews of these relationships have been contributed to the present meeting by Goyer and Rhyne (1973) and Shibko et al. (1975) and other literature includes Averill and King (1926), McCance and Widdowson (1935), Maley and Mellor (1950), Vohra et al. (1965), Oberleas et al. (1966), Evans (1973), Trowell (1973), Davis and Nightingale (1974), Klevay (1975a, 1975b), Reinhold (1975), and Dacre and Ter Haar (1975).

Factors to be considered in evaluating the bioavailability of an element in a diet also include the physico-chemical form and properties of the compound, the site of absorption, the surface area involved, the blood supply to the site of absorption, and other membrane transport factors. Although these factors may be tested only in model systems, they may be important in the evaluation of the absorption of particulate inorganic matter following inhalation and subsequent swallowing, such as might occur in an industrial situation. In addition, as in the case of toxic elements normally present in food, key factors affecting bioavailability are mineral interaction and dietary components.

IV.3.2.1.2.1 Cadmium

On the basis of data on human beings (Rahola et al., 1972) it may be concluded (TGMA, 1973) that approximately 6% of an oral dose of cadmium is retained after 10-15 days. Data by Kitamura (1972) and Yamagata et al. (1974) as well as by Rahola et al. (1973) are in accord with this conclusion. Observations discussed at the present meeting make it reasonable to conclude that this figure may vary depending on such factors as calcium and protein content of the diet, etc., between 3 and 10% (Friberg et al., 1974).

Recent data from experiments on rats (Stowe et al., 1974) suggest that the dietary concentration of pyridoxine (vitamin B_6) will influence cadmium absorption. In young mice the absorption rate of cadmium was found to be higher than in adult mice (Matsusaka et al., 1972), the whole-body retention being about 10 and 1%, respectively, 2 weeks after an oral dose of radioactive cadmium. Age might also be meaningful for the gastrointestinal absorption of cadmium in human beings.

IV.3.2.1.2.2 Lead

TGMA (1973) concluded that the gastrointestinal absorption of lead compounds in adult human beings is between 5 and

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[x] It is well recognized that there are both inorganic and organic (e.g. tetraethyl-) lead compounds of toxicological importance. However, because of the considerably greater ubiquity of the inorganic compounds, these were the only ones considered during the previous meeting in Buenos Aires (TGMA, 1973). In the present report data concerning lead will refer to inorganic lead compounds unless otherwise explicitly stated.

15%. This figure may be greatly altered by components in the diet, age and whether the subject ingesting lead is in the fasting state or not. The absorption of lead was shown to be as high as 50% in fasting human subjects while in non-fasting subjects it was 6-14% (Wetherill et al., 1974). Alexander et al. (1973) reported that a group of children whose ages ranged from 9 months to eight and a half years absorbed 50% of the dietary lead intake. Studies on experimental animals have shown that other dietary compounds, e.g. phytate, protein and lactose may have significant effects on the absorption of lead (see section IV.3.3.1.2).

IV.3.2.1.2.3 Mercury[x]

TGMA (1973) considered that the absorption of inorganic mercury was at least 7% on the average. This figure will be valid for inorganic salts of mercury (II). As for the absorption of elemental mercury introduced into the gastro-intestinal tract, it is extremely limited, probably amounting to less than 0.01% of the administered dose. The absorption of methylmercuric compounds is about 95% of the administered dose in human beings, irrespective of whether the methylmercuric radical is bound to protein or administered as a water solution of a salt.

IV.3.2.1.3 Percutaneous absorption

The skin can be an important route by which metals enter the body, especially for organometallic compounds and metal salts of fatty acids. This type of absorption has been dealt with at a meeting of the Subcommittee on the Toxicology of Metals (Wahlberg, in Dukes and Friberg, 1971) and will not be discussed further in this report.

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[x] The different forms of mercury will be classified as elemental, inorganic and organic. Elemental mercury is the unoxidized element which usually exists as liquid or vapor. Inorganic compounds include both monovalent (mercury (I) or mercurous) and divalent (mercury (II) or mercuric) mercury salts and include also complexes and chelates with organic ligands in which mercury is reversibly bound. For the sake of simplicity the term inorganic will refer to mercuric compounds unless specified as mercurous. Those compounds in which mercury is directly linked to a carbon atom by a covalent bond will be classified as organomercurial compounds or organic mercury compounds.

IV.3.2.1.4 Transfer of metals via placenta and maternal milk

Another route of absorption is the transplacental transfer, which gives an initial body burden. Fetuses should be considered as an especially susceptible group. If transplacental transfer of a particular metal is high, fetal exposure may be a significant factor for the assessment of the total exposure. Yet another route of absorption which may be of consequence in infants is transmission via mother's milk. These types of exposure were discussed previously (TGMA, 1973).

There is evidence that the human placenta is freely permeable to certain metal compounds and that some metals are secreted in maternal milk during the perinatal period. It is during this period that growth, especially of the central nervous system, is most rapid. Direct assimilation of metal by the fetus and neonate may be especially important from the viewpoint of age-related susceptibility factors. For example, in the case of methylmercury compounds, transplacental transfer results in higher blood levels in the infant at birth than in the mother (Tejning, 1970; Suzuki et al., 1971; Bakir et al., 1973). Transmission via milk has been shown to contribute significantly to infant blood levels of methylmercury (Amin Zaki et al., 1974).

IV.3.2.2 Transport, distribution and excretion

IV.3.2.2.1 Transport and distribution

An outline of general principles of transport and distribution of metals in the body has been discussed previously (TGMA, 1973). It was concluded that the binding of a metal to plasma proteins is of great importance for its distribution. The portion of the metal bound to the "diffusible" fraction plays an especially prominent role in the exchange of metals through biological membranes and in the subsequent translocation to critical body organs. Special comment was made on metal accumulation in organ systems with specialized functions, such as the kidney and the brain. Quantitative data on the proportion of metal transferred to the critical organ will be given in section IV.3.4.

IV.3.2.2.2 Excretion

General aspects of the gastrointestinal excretion as well as the renal excretion of metals have been reviewed, including such topics as the mechanism of these two types of excretion and the factors governing them. Specific information included the relative participation of various mechanisms in the excretion of different metals, and where possible, also the quantitative relations to the total body burden (TGMA, 1973).

In addition to the mentioned aspects, the renal excretion was discussed in relation to the functional impairment of organs as well as the excretion of metals via the mammary gland. These data are basic to the understanding of the metabolic models for the various metals and to the understanding of the use of excretion media as indicators (or indices) of exposure and/or accumulation. Since such data are not indispensable for dose-response evaluations and calculations, they will not be given in detail in this report.

IV.3.2.3 Accumulation and retention in critical organs

IV.3.2.3.1 General principles

An organ accumulates metal when uptake exceeds elimination; a steady state is reached when uptake equals elimination. A common way to express the elimination rate is in terms of biological half-time, a concept which is only meaningful when the elimination can be approximated with a reasonable accuracy to a single exponential first order function. The concentration in the organ at any time then can be expressed by the following equation:

$$C = C_0 \cdot e^{-bt} \qquad (1)$$

C = concentration in the organ at time t
C_0= concentration in the organ at time 0
b = elimination constant
t = time

The relation between the elimination constant and the biological half-time is as follows:

$$T = \frac{\ln 2}{b} \qquad (2)$$

T = biological half-time
$\ln 2$ = natural logarithm of 2 = 0.693.

The exponential function is not always representative of the elimination of toxic metals; therefore the equations should be treated with caution.

Of pertinence for toxicological evaluation is the highest concentration reached in the critical organ during continuous exposure. A simple model, based on the assumption that metal turnover occurs according to the above mentioned retention equation (1), that the rate of uptake of the metal is faster than the elimination rate, and that a constant fraction of the intake is taken up by the organ, will give the following expression for the amount accumulated in the critical organ during continuous exposure:

$$A = \frac{a}{b}(1 - e^{-bt}) \qquad (3)$$

A = accumulated amount
a = fraction of daily intake taken up by the organ
b = elimination constant
t = time of exposure

At steady state:

$$A = \frac{a}{b}$$

The above mentioned mathematical expressions were worked out by the TGMA (1973), and to reiterate, they are based on the assumption that intake and absorption are constant. If either of these changes systematically with age, type of exposure, period in time, etc., the equation must be modified.

If the elimination rate represents elimination from more than one compartment, it will be necessary to have a multifunctional mathematical expression. In such cases, multiple biological half-times or multiple elimination rates must be calculated from available data.

For most metals, the assumption that all of their movement in the body is dependent only on the concentration gradient is an oversimplification. Active transport or metabolic processes are often involved in metal turnovers. Consequently, it has been recognized that this simple model generally does not give a sufficiently accurate description of the concentration in the critical organ. Specific data as to the accumulation of various metals in tissues as well as numerical values for fractions in critical organs and biological half-times must be presented to broaden the possibilities of the model, as was done by TGMA (1973).

IV.3.2.3.2 Cadmium

A simple model implying that a constant fraction of absorbed cadmium is directly transferred to the kidney may not be adequate to arrive at the renal accumulation of cadmium, since a part of the transfer probably occurs slowly via the liver (Nordberg, 1972b). Since to date no other models have been introduced, the simple model might at least provide an approximation. Interpretations of such approximations of course must be made with caution.

Using the simple model, biological half-times for the kidney and kidney cortex between 17.6 years (Tsuchiya and Sugita, 1971) and 38 years (Kjellström, 1971; Kjellström et al., 1971; Friberg et al., 1974) have been estimated, based on available information on the accumulation of cadmium in cross-sectional samples of Japanese and U.S. populations.

The fraction of whole-body cadmium that will be deposited in the kidney varies depending on the time after administration. Autopsy material from a normal population at age 50 suggests that 1/3 of the body burden is located in the kidneys. On higher exposure (industrial) the fraction of the body burden that is found in the kidney is smaller than 1/3 (Friberg et al., 1974).

Using the model described by equation (3) and correcting it for systematic changes in caloric intake and kidney weight during lifetime, it has been calculated that the cadmium concentration in kidney cortex at 50 years of age would be 50 µg/g following a daily intake of 24 ng/calorie (65 µg/day for an adult) assuming about 1.7% (1/3 of 5%) deposition of body intake in the kidney (Friberg et al., 1974). Further data from calculations based on these assumptions will be found in section IV.5.2.2.

IV.3.2.3.3 Lead

The major complication in establishing a mathematical model for lead absorption or retention is the strong affinity of lead for bone. The estimates of half-time for lead in bone have varied from 64 days in the spine of rats to 7,500 days in the skeleton of a dog. The biological half-time for lead in bones of humans has been estimated to be 10 years (TGMA, 1973).

It has been thought that there are at least four bodily compartments for lead. The retention of lead has been treated as a power function model rather than an exponential model in some publications. It was concluded during the Buenos Aires meeting that lead required a particularly complex metabolic model.

Since that time, Rabinowitz et al. (1973) have introduced a new technique for measuring lead metabolism, by administration of a stable isotope to human subjects and determination of isotope ratio and lead concentration in indicator (or index) media. The results fit a 3-compartment mathematical model. Compartment one is blood lead, possibly having a rapidly exchanging soft tissue component, and has an estimated mean life[*] of 27 days (corresponding to a biological half-time (T) of 18.7 days). A second compartment consists of a more stable soft tissue component and the rapidly exchangeable fraction in bone with an estimated mean life of 30 days (corresponding to T = 20.8 days). The third compartment is the stable fraction in bone with a much longer mean life of almost 30 years (T = 20.8 years). This is compatible with earlier estimates of half-time of about 10 years. These data are derived from studies of two normal male adults without abnormal exposure to lead.

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[*] Mean life = $\frac{T}{\ln 2}$ or = $\frac{1}{b}$

IV.3.2.3.4 Mercury

The different forms of mercury exhibit different kinetics as regards distribution in the body. Distinction will be made between elemental mercury, inorganic mercury compounds, and various organomercury compounds.

IV.3.2.3.4.1 Mercury vapor

TGMA (1973) concluded that quantitative data were not sufficient to be entered into a model for mercury accumulation in the human brain after vapor exposure. The biological half-time in the brain has been shown to be longer than that in other organs in animal experiments, but it is not possible to give exact figures. Certain areas of the brain especially retain mercury (TGMA, 1973). Data from Takahata et al. (1970) indicate a long biological half-time, at least some years, for mercury in the brain of human beings after vapor exposure. A discussion of the biological half-time of mercury in the brain, including how to interpret animal data when modelling the human situation is available (Berlin, M. 1975); in rats approximately one percent of the absorbed dose was present in the brain in a short-term experiment. No corresponding percentage has been established for human beings.

Mercury vapor (Hg^o) is oxidized to Hg^{++} in blood (Clarkson et al., 1961). However, the delay after inhalation allows some mercury vapor to remain dissolved in the blood stream for sufficient time to reach the blood-brain barrier (Magos, 1967, 1968). This explains why, after vapor exposure, the brain takes up more mercury than after the injection of equivalent doses of inorganic mercury salts (Berlin, M. et al., 1966, 1969; Nordberg and Serenius, 1969). Approximately 80% of the inhaled dose is retained in man after a brief exposure (Teisinger and Fiserova-Bergerova, 1964; Nielsen-Kudsk, 1965a). The uptake of mercury in man (Nielsen-

Kudsk, 1965b) and rats (Magos et al., 1973) is decreased by ethanol. Amino-triazole has the same effect on rats (Magos et al., 1974a). Because these agents increase the exhalation of Hg^{0} from rats injected i.v. with elemental mercury, it is clear that this effect is due to the inhibition of the oxidation of Hg^{0} to Hg^{++} (Magos et al., 1973; Magos et al., 1974a).

Differences in the distribution of mercury between pretreated and control groups indicated that oxidation takes place not only in the blood but also in other tissues. Studies in vitro using blood as a model system showed that catalase is the enzyme mainly responsible for the oxidation of Hg^{0} (Nielsen-Kudsk, 1973; Magos et al., 1974a). Experiments carried out with acatalasemic mice confirmed this finding (Sugata and Clarkson, unpublished data).

IV.3.2.3.4.2 Inorganic mercury compounds

The biological half-time of mercury in the whole body of humans given mercury (II) orally has been found to be 29-41 days for 5 women and 32-60 days for 5 men (Rahola et al., 1972; Miettinen, 1973). About 7% of the total administered dose was excreted with this half-time. This figure involved a minimum absorption figure (TGMA, 1973). According to data from animal experiments it may be concluded that the half-time in the kidney is somewhat longer than that in the whole body (Rothstein and Hayes, 1960, and others; review: Nordberg and Skerfving, 1972). The retention of mercury in the kidney of animals is shorter than in the brain (Berlin, M. and Ullberg, 1963; review: Nordberg and Skerfving, 1972). The rather complicated, multicompartmental behavior of the kidney on different types of exposure to mercury is still poorly understood. At low dose levels a rather large amount of total mercury elimination occurs via bile and feces. With increasing administered dose, a larger part of the absorbed amount is eliminated by urine, as shown for rats (Rothstein and Hayes, 1960) and man (Bürgi, 1906).

IV.3.2.3.4.3 Organomercury compounds

The short-chain alkylmercurials (methyl, ethyl) possess kinetics markedly different from those of other organomercurials. They will therefore be treated separately.

IV.3.2.3.4.3.1 Methylmercuric compounds

The metabolism of methylmercuric compounds is sufficiently well known to allow adequate calculations of accumulation. Data from studies in animals and man justify the assumption that a relationship exists between the concentration in each organ and the total body burden of methylmercury, and that a relationship exists among the levels of methylmercury in the different organs (Berglund et al., 1971; Nordberg and Skerfving, 1972). Elimination is correlated with the total body burden, i.e. a definite fraction of this is eliminated per unit of time. The course of elimination in continuous exposure thus can be expressed according to equation (3). Reasonably accurate calculations should be possible for the brain content as long as no signs of toxicity occur. A higher brain/blood quotient in monkeys after neurological damage than in monkeys not exhibiting signs of toxicity has been shown by Berlin, M. (1975). This implies that at the time neurological damage takes place, the distribution pattern of methylmercury changes. This point will be discussed further in the section on dose-response relationships.

Data on the biological half-time in the brain of humans given tracer doses do not suggest that it would be different from that in the body as a whole (Åberg et al., 1969; Berglund et al., 1971). The latter has been estimated to be about 70 days on the basis of both human experimental and epidemiological data. In nine male and six female volunteers given tracer amounts of methylmercury, the half-time for whole-body clearance ranged from 52 to 93 days, with a mean value of 76 days (Miettinen, 1973). An even more substantial variation in biological half-time was found in blood by Skerfving (1973), ranging from 58 to 164 days in 5 persons consuming $MeHg^+$ from fish. One person had 164 days and significantly differed from the others (58-87 days). Bakir et al. (1973) found a range of 45-105 days and a mean of 65 days for mercury in the blood of 16 persons in the Iraqi outbreak.

Recent data from Al-Shahristani and Shihab (1974), based on sequential analysis of mercury in hair from 48 persons poisoned by methylmercury compounds in Iraq, show a variation in the half-time from 35 to 189 days, with a mean value of 72 days. The distribution curve for the biological half-time in these 48 persons had two distinct peaks, one ranging from 35 to 100 days which represented about 90% of the group and the other ranging from 110 to 120 days. This finding and that of Skerfving (1973) suggest that a deviant population might exist who have a longer elimination time for $MeHg^+$.

Using tracer doses of a radioactive methylmercuric compound (Åberg et al., 1969), it was found that the amount of radioactivity in the head was 10% of the total body burden. Since the brain concentration substantially exceeds that in other tissues of the head, it can be assumed that the amount present in the brain is about 90% of the total head burden.

Taking the formula (3) and a biological half-time of 70 days, calculations at steady state in the brain have been made which show a concentration of approximately 1.5 µg Hg/g brain tissue at a daily absorption of 4 µg Hg/kg body weight in the form of a methylmercuric compound (Figures on steady state brain concentrations at a certain intake level were erroneously stated in the report by TGMA (1973).

Biotransformation of methylmercuric compounds, resulting in the release of inorganic mercury, has been demonstrated in different animal species (review: Nordberg and Skerfving, 1972) and also in man (Sumino, 1973; Bakir et al., 1973). The mechanism of this biotransformation is unknown. The concentration of inorganic mercury in the brain is low, that is, less than 10%, relative to methylmercury. In whole blood the relative amount of inorganic mercury is low, which is due to the difference in red-cell-to-plasma distribution of inorganic mercury and methylmercury. The relative amount of inorganic mercury in plasma is high while almost all mercury in the red cells is in the organic form. From 25-85% of the mercury in bile was found to be inorganic in the squirrel monkey (Berlin, M. et al., 1975a) and 40% was inorganic in human mothers' milk (Bakir et al.,1973). The urine also contains a relatively high proportion of inorganic mercury.

IV.3.2.3.4.3.2 Ethylmercuric compounds

Suzuki et al. (1973) have reported on the metabolic fate of an ethylmercuric compound. Five patients accidentally received human plasma infusion containing ethylmercurithiosalicylate. The mercury in red cells from these patients was mainly in an ethylmercuric form; in contrast that in plasma and urine was inorganic. Only the former showed a rapid decrease with time (half-time: about 10 days). Observations on autopsy tissue from one patient revealed that 8.6% of the administered dose was recovered from the brain.

IV.3.2.4.3.3 Other organomercury compounds

The other organomercurials of practical importance are the aryl and alkoxyalkyl mercurials such as phenyl- and methoxyethylmercury compounds. Studies on experimental animals indicate that these compounds are rapidly converted to inorganic mercury in the body tissues. Within a few days after a single administered dose, the organmercurial has disappeared and the distribution in tissues closely approaches that of inorganic mercury (review: Nordberg and Skerfving, 1972).

IV.3.3 Metal concentrations in biological media as indicators (or indices) of exposure and of concentrations in critical organs

The following general aspects have been presented previously (TGMA, 1973). In assessing the effects and dose-response relationships connected with the accumulation of metals, especially those with long biological half-times, it is often desirable to use concentrations of metals in blood, urine or hair. When steady state is reached, a constant ratio is held between blood and tissue concentrations of the metal, and except for metabolic variations, there is also a relationship to the urinary concentration of the metal. For a biological material to be a good indicator of accumulation, especially for substances with long biological half-times, it is not enough that the relation should be constant at steady state conditions; it should also be reasonably constant during the period of accumulation. Blood, which transports most absorbed metals, is not necessarily a good indicator of accumulation in the critical organ.

IV.3.3.1 Cadmium

The usefulness of various biological materials as indicators (or indices) of exposure and retention has recently been discussed (Friberg et al., 1974; Nomiyama et al., 1975a). TGMA (1973) and Friberg et al. (1974) considered that cadmium concentrations in urine during continuous exposure would increase sharply with the advent of renal tubular impairment. Nomiyama et al. (1974) and Nomiyama and Nomiyama (1975) found an increase after long-term administration of high levels of cadmium to experimental animals per os.

A sharp increase in urinary concentrations of cadmium in connection with the appearance of morphological changes in the renal tubules of rats has been reported (Kawai et al., 1975). In mice, Nordberg (1972a, 1972c) showed that during the early phase of exposure, before renal tubular impairment occurred, a correlation existed between the body burden and urinary concentration of cadmium. Tsuchiya et al. (1972a, 1972b), in studies on normal Japanese not excessively exposed to cadmium, estimated a biological half-time of 13 years for the body compartment reflected in urinary excretion, which is not widely different from the estimated biological half-time in the liver (7 years) and kidney (17.6 years) in the same study. These results support the evidence of a relationship on a group basis between urinary excretion and accumulation in the main storage organs for cadmium in the body during the phase when there is no renal tubular impairment (TGMA, 1973). Due to a substantial interindividual variation, individual values may not always lend themselves to estimates of body burdens of cadmium.

Conclusive evidence on the relation of blood values of cadmium to the concentration in the kidneys has not been presented. Available evidence from animal experiments and human data has been compiled and discussed elsewhere (Friberg et al., 1974). It was concluded that blood values probably do not reflect kidney accumulation of cadmium but may reflect the most recent exposure.

IV.3.3.2 Lead

Except under controlled experimental conditions the dose cannot be measured exactly, but lead concentrations in various biological media may be used as an estimate of dose. These are indirect measures, or in other words indicators of dose.

Blood lead levels are the most reliable measurement of recent exposure and particularly useful for epidemiological studies. Interpretation of blood lead in the individual must take into account the following considerations. This indicator reflects a dynamic balance between exposure, amounts in tissue, and excretion. Blood lead level, however, reflects only one point in time and it may reflect a dose which is steady, increasing, or decreasing. Lead levels of critical organs such as brain, bone marrow and kidney probably relate closely to effects in these organs, but they cannot be measured readily. For this reason, studies relating lead concentrations in blood, urine, and other biological samples with those in the critical organs are lacking while data relating the blood lead levels to current exposure are abundant. There is a long-standing debate as to whether blood lead levels should be corrected for hematocrit values. About 90% or more of blood lead is in red blood cells; therefore care should be taken to assure that the sample is homogeneous.

Urinary lead may also reflect recent exposure but is more highly variable than blood lead. One method for estimating mobile tissue lead is the measurement of urinary excretion following EDTA administration. In studies on children and adolescents (Chisolm et al., 1975) a statistically significant linear relationship was found between blood lead concentration and the logarithm of the quantity of lead excreted in the 24-hour period immediately following administration of calcium EDTA in a dose of 25 mg/kg. Thus, the mobile fraction of the body lead burden increases exponentially as blood lead concentration increases arithmetically in the range of 8-75 µg Pb/100 g blood.

Further investigation with regard to dose and rate of administration of calcium EDTA may provide better standardization of this test for estimating the chelatable or mobile portion of the body lead. This may serve as a more reliable "chemical biopsy" of the concentration of lead in critical organs in individual humans than single measurements of lead in blood or urine.

Lead levels in hair may reflect long-term exposure but because of the problem of external contamination, these may not be a good indicator of body burden (TGMA, 1973). Increased concentration of lead in teeth persists even after blood lead levels have decreased (Albert et al., 1974).

It has been suggested on the basis of studies in humans (Needleman and Shapiro, 1974) and baboons (Albert et al., 1974) that the lead concentration in dentine of shed deciduous teeth is a good indicator of past exposure to lead during infancy and early childhood. Factors such as time of mineralization of the tooth, its status in regard to caries, resorption of the root, localization of the dentine analyzed, and the method of sampling have to be taken into consideration when interpreting data about dentine lead content.

For estimating total body burden, bone lead concentration is most accurate but is largely inaccessible except at autopsy If bone is biopsied, care should be taken to distinguish between membranous (cortical) and cancellous (trabecular) bone.

IV.3.3.3 Mercury

IV.3.3.3.1 Mercury vapor

Correlations have been established between exposure and blood or urine values in studies of groups of workers (Smith et al., 1970). Urine values for individuals are useful as an indicator (or index) of exposure only when serial monitoring is carried out (Suzuki et al., 1968).

No evidence has been obtained on the relation between blood and brain (critical organ for prolonged exposures) values in humans. In individual cases, blood or urine values may give conflicting conclusions about the risks for poisoning.

IV.3.3.3.2 Inorganic mercury compounds

There is not a constant ratio between mercury concentration in blood and kidney (critical organ) according to animal experiments (Berlin, 1963; review: Nordberg and Skerfving, 1972). Studies in man by Miettinen (1972, 1973) following a single oral tracer dose showed that the biological half-time in blood (20 days) is half of that in the entire body (40 days). It seems probable that blood is not a good index for evaluation of mercury accumulation in kidney. Animal experiments do not support urine mercury as a good index of kidney concentration (TGMA, 1973).

IV.3.3.3.3 Organomercury compounds

IV.3.3.3.3.1 Methylmercuric compounds

Animal experiments have shown that blood concentrations of mercury correlate with mercury concentrations in the brain after exposure to methylmercuric compounds, and that these parameters are linearly correlated with the absorbed amount of $MeHg^+$ up to dose levels at which clinical signs occur (review: Nordberg and Skerfving, 1972). In epidemiological studies, a linear correlation between dose measured as daily intake of $MeHg^+$, and mercury level in blood and hair (Bakir et al., 1973; Skerfving, 1974; Kazantzis, 1975; Suzuki, 1975) has been observed. Thus, at dose levels of $MeHg^+$ not associated with signs or symptoms of poisoning, there is good evidence that blood concentrations of mercury reflect the $MeHg^+$ concentration in the brain. At dose levels of $MeHg^+$ causing toxic manifestations, animal experiments have shown that mercury concentrations in blood and brain rise more rapidly with cumulative daily dose than at lower body burdens of $MeHg^+$. Epidemiological data from Iraq (Bakir et al., 1973) suggest that this may also be the case in man.

Available evidence indicates a rectilinear relation between total mercury levels in blood and hair, the hair levels being about 300 times higher than the whole blood levels for $MeHg^+$ (Berglund et al., 1971; Nordberg and Skerfving, 1972). These conditions make hair levels a good indicator of body or brain accumulation of mercury. Standards for type and length of hair to be clipped must be set up. The delay in the appearance of mercury in hair can be a drawback, but it can also be useful. With knowledge about the growth rate of hair, the course of past exposures can be evaluated (Tsubaki, 1968; Al-Shahristani and Shihab, 1974). Several factors may lead to errors in the interpretation of mercury levels in hair:

(1) Hair collected from different sites of the body has different growth rates.

(2) External contamination may result from occupational exposure and from the use of cosmetic and pharmaceutical preparations. The possibility of external contamination from mercury in sweat needs investigation.

(3) When hair samples are used to recapitulate brief, high level exposures such as in the Iraqi outbreak, corrections must be made for differences in growth rates of individual strands of hair and for accidental displacement of the strands during collection and transportation (Giovanoli and Berg, 1974).

Urinary values of methylmercuric compounds do not have practical significance because of the low levels of $MeHg^+$ in urine, and the concomitant presence of inorganic mercury. Total mercury in urine therefore is not a reliable measurement of body burden of methylmercuric compounds.

IV.3.3.3.3.2 Ethylmercuric compounds

Because of the limitations of available data, concentrations in blood, hair, and urine relevant to exposure cannot be fully discussed. Except for the elevated rate of release of inorganic mercury which has been shown in the mouse, rat and man (Takeda and Ukita, 1970; Suzuki et al., 1973), ethylmercuric compounds display a similarity to methylmercuric compounds in their kinetics. For this reason, the remarks on indicator (or index) media for methylmercuric compounds probably apply to ethylmercuric compounds as well.

IV.3.3.3.3.3 Other organomercury compounds

Concerning organomercurial compounds other than the alkylmercuric ones, it is assumed that both urinary excretion data and blood values, when used on a group basis, might provide information about the exposure (review: Nordberg and Skerfving, 1972).

IV.4 EFFECTS

IV.4.1 General considerations

When a toxic metal compound contacts a cell or tissue, binding occurs to various ligands in that tissue. This binding may be defined as an effect, but may or may not harm the function of the cell. A small number of metal-containing molecules usually do not influence the function of the cell, or there may exist a subcritical effect, i.e. an influence which is not adverse. When larger numbers of molecules are bound, the degree of effect depends on the function of the binding ligands.

A functional change may occur if the response at the molecular level involves a certain number of binding sites. As defined earlier, the critical concentration in a cell is reached when the functional change becomes adverse. Only a few studies on critical cell concentrations have been reported probably because of the difficulties in conforming to adequate model systems and the extreme difficulties in studying critical effects on individual cells in a living animal.

Theoretically, individual cells can be observed in microscopic investigations, but if cellular injury does not include specific morphological features, it is often difficult to assess whether changes in individual cells are induced by metals. A small number of cells normally undergo degeneration. It is not easy, at present, to correlate morphological findings with concentration of metals in individual cells. A further discussion will be given in sections IV.4.1.2 and IV.5.1.

Granted the present state of knowledge concerning metal toxicity, a more viable entity for discussion is the critical organ concentration. Specific quantitative data for critical organ concentrations for various metals will be discussed in Section IV.5; also, relationships between concentrations of metals in indicator (or index) media and effects will be given there.

In this section, a general outline of the types of changes that may be induced by metals will be given, and it will also be discussed how they can be assessed by clinical observation, functional tests, and morphological and biochemical techniques. It will be attempted to draw general conclusions concerning the pathogenetic mechanisms by which metals exert their effects in biological tissues. Types of effects evidenced for various metals, especially cadmium, mercury, and lead, in experimental animals and exposed human beings will also be reviewed. An appraisal as to which of these effects may be regarded as the critical effect in human beings during different types of exposure situations will be made.

IV.4.1.1 General aspects of symptoms, signs, and other changes assessable by functional tests

Two kinds of effects are symptoms and signs. By symptoms are meant here complaints of individuals; signs are observed by examinations and tests. Both symptoms and signs can be induced by exposures to metals. Metals may interact with other substances to give a certain clinical syndrome. They may also interact with each other, thus modifying their per se effects. Standardized classification of functional disturbances at the physiological and clinical levels is a prerequisite for quantitative evaluation of such effects. This is particularly true for case studies and epidemiological work, which constitute the foremost approaches for evaluating clinical manifestations of effect.

Some metals give rise to an immediate, local effect at site of absorption, whereas others give systemic effects a considerable time after absorption has taken place. A clinical effect after repeated exposures may be a result of accumulation of metal, reaching a certain critical concentration, or sometimes a result of repeated subclinical damage. In the specific part of section IV.4 to follow (sections IV.4.2, IV.4.3, and IV.4.4), the pathogenic mechanisms for clinical manifestations of metal toxicities will be delved into. An attempt will be made to place the types of damage in the mentioned categories.

The clinical effect may be followed by complete restitution, but chronic functional changes may also result. The relation between biochemical alterations and clinical observations is of great interest, especially when latency periods and secondary effects enter into the picture.

IV.4.1.2 General aspects of morphological changes at various structural levels

Metals may give rise to gross structural macroscopically evident organ damage, light microscopical morphological changes as well as to ultrastructural ones. These various types of damage may be observed both in laboratory animals and human beings.

A compelling topic is how morphological observations relate to biochemical and functional ones. It may be argued that any biochemical change involves changes in molecular configuration and therefore structural change. Thus, the debate on which comes first, the biochemical or the morphological change, is artificial; the decisive factor is actually the resolving power of biochemical analysis versus morphological analysis. For some types of injury such as permeability changes in membranes, there is often ultrastructural change discernible by transmission electronmicroscopy (e.g., for metals like lead, mercury and cadmium). Interaction with mitochondrial membranes usually results in change in mitochondrial configurations. Combinations of data concerning clinical observations, light microscopical and ultrastructural, as well as biochemical changes may give interesting indication on the mechanism by which metals exert their effects on tissues.

Conceptually, there may be two mechanisms for producing a change observable by morphological techniques. One mechanism is metal interaction with membranes in the cell in such a way as to bring changes in the function and integrity of intracellular organelles such as mitochondria. Such changes, when known to reflect cellular degeneration, may be regarded as adverse effects. The other mechanism behind morphological change is the binding of the metal to certain ligands in the cell, these tissue-metal complexes becoming visible by morphological techniques. Such metal-tissue binding may or may not interfere with the structural and functional integrity of the cell. Depending on its nature, such an effect may or may not be regarded as an adverse effect in the cell. Sometimes one morphological change alone (e.g., lead inclusion bodies) enables a specific diagnosis of the kind of metal exposure. In most cases a combination of observations is necessary to ascertain that one given change has indeed been induced by metals. This is especially true of the type of morphological change that is nonspecific.

Single or repeated exposures may give quite different types of morphological changes. Usually more subtle morphological changes are reversible, whereas necrotic tissues represent nonreversible changes. Some individual necrotic cells may be replaced by regeneration. Direct and delayed effects may have specific morphological features and their relation to biochemical modifications could be a fruitful topic for discussion and research.

Morphological aspects of changes arising as a result of accumulation of damage versus damage from accumulation of metals are noteworthy. Sometimes interactions of various effects may be explainable on the basis of morphological features.

A specific type of structural effect, chromosomal aberrations, can be induced by metals and metal compounds (e.g. methylmercury). These aberrations reflect interference with the genetic material of the cells, but their relation to somatic effects in the individual or his offspring is difficult to assess. Carcinogenic and cocarcinogenic effects can also be induced by metals. Such effects are usually assessed by long-term experiments on animals or by statistical evaluation of observations on man. Much attention has recently been drawn to structural effects of various chemicals, including metals and metal compounds, that are induced during embryogenesis and referred to as teratogenic effects.

IV.4.1.3 General aspects of biochemical changes

Biochemical approaches are indispensable for our understanding of the effects of toxic metals and other toxic substances. Many avenues of approach have opened with modern technology and new knowledge, especially in molecular biology, e.g., the role of cAMP. These avenues remain to be explored in greater depth. This review cannot give an exhaustive treatment on biochemical effects, but has been limited to some examples. Specific data on biochemical modifications that may constitute the basis for signs of symptoms of adverse effects will be given in later sections. It should be pointed out that the knowledge of biochemical changes is limited, and most of the results have been obtained with in vitro experiments.

IV.4.1.3.1 Binding with proteins and other ligands

Binding of different metals with different ligands may constitute the primary effect at the biochemical level, accounting for the many effects of metals on cell membranes, enzymes, and other cellular constituents. For example, lead, mercury, and cadmium show a strong affinity for ligands such as sulfhydryl groups, phosphates, cysteinyl and histidyl side-chains of proteins, purines, pteridines, and porphyrins, and can therefore act at a large number of biochemical sites. In addition, metals may interact with many other ligands, such as hydroxyl, carboxyl, amino and other groups. The interference with specific biochemical activities will depend on the type of ligand and the structural and functional organization of these ligands, e.g., whether active binding sites are affected or not. General aspects on the biochemical background for functional impairment induced by toxic metals have been given elsewhere (Singerman, 1972; Vallee and Ulmer, 1972; Sigel, 1974).

IV.4.1.3.2 Effects on the cell membrane

The affinity of metals for cell membranes results in a particular susceptibility of membrane functions. The cell membrane is considered to be an active part of the cell, controlling the transfer of substances into and out of the cell by means of exchange mechanisms for active transport or carrier mechanisms for passive transport. The essential elements of the carrier mechanism are the reactions of a transport substrate with a membrane component to form a complex, which then moves from one side of the membrane to the other where the substrate is released. The structure of cell membranes contains large quantities of lipids, and,since metals may bind strongly to the cell membrane, this may lead to changes of the surface active properties of the lipid. These and other changes may further lead to alterations of membrane permeability and metabolic activities of surface enzymes. The effect on activity at the cell membrane may be proportionately low compared to the amount of metal present, in that the metal may bind in large proportion to inactive sites of the membrane.

IV.4.1.3.3 Effects on enzymes

Metals may inhibit the enzymes of the membrane, resulting in modifications of the utilization of nutritionally-required external substrates, the active transport mechanism and the synthesis of membrane constituents. Obviously, the effect on enzymes may also occur wherever they are present, including intra- and extracellular locations. Reduction in enzyme activity depends upon the accessibility of metal-binding groups. For example, it has been shown *in vitro* that lead, mercury, and cadmium inhibit a large number of enzymes having functional sulfhydryl groups. Additional important binding sites on enzymes include the carboxyl groups of glutamic and aspartic acid and the epsilon aminoacid group of lysine. Some enzymes, the so-called metalloenzymes, contain a metal which is bound in such a specific manner that it cannot be removed without loss of enzyme activity. Toxic metals may displace the essential metals from such metalloenzymes with resulting loss in enzyme activity. In some cases the new metalloenzyme may retain activity, e.g., cadmium and lead carboxypeptidases have been shown to have appreciable esterase activity (Vallee and Ulmer, 1972). The kinetics of enzyme inhibition has provided basic information on the probable nature of the action of metals at the molecular level. In addition to the interaction of metals with enzymes per se, the rate of enzymic reactions may be reduced by binding of metals with coenzymes or substrates. The observable effects of enzyme inhibition may be on such functions as synthesis of molecules such as heme, or basic bioenergetics.

When the metal has affected the cell permeability or cellular structures, such as lysosomes, there may be an increase of enzymes in intracellular or extracellular fluids, but metal interactions may also directly "activate" some enzymes.

IV.4.1.3.4 Effects on the energy metabolism system

The chemical reactions providing the energy for the various functions of the cell are mediated by enzyme systems, but also require high energy nucleotides. Lead, mercury, and cadmium disrupt pathways of oxidative phosphorylation, a process associated with the integrity of the mitochondrial membrane; the precise effect depends upon the individual properties of the metal. Lead forms complexes with the phosphate groups of nucleotides and catalyzes a nonenzymatic hydrolysis of nucleosidetriphosphates, particularly ATP (Rosenthal et al., 1966a, 1966b). It may also interfere with ATP synthesis when an energy dependent mitochondrial lead uptake occurs (Walton, 1973).

IV.4.1.3.5 Effects on nonenzymic molecules

Metals may cause structural changes in proteins resulting in denaturation, alteration of bioelectric properties, impairment of the transmission of nerve impulses, and loss of transport or other vital functions. The activity of very large molecules, such as hemoglobin, intermediate-sized molecules such as metallothionein, and small molecules important for their redox potential, such as ascorbic acid or glutathione, may all be affected by toxic metals. Lead, mercury and cadmium all bind to nucleic acids affecting the configuration of these essential molecules. Those metals which are transition elements may form many different complexes in biological systems on account of their changes in oxidative state. While lead, mercury, and cadmium are not transition metals, they do have strong tendencies to complex formations.

IV.4.1.3.6 Other biochemical considerations

It should be clear that metals may interact with cells in many ways, including the membranes, organelles, metabolic and mitotic systems. The cell is a complex organism with its own internal homeostatic mechanisms, and its function as a whole should not be forgotten when investigating biochemical mechanisms at the molecular level. Similarly, the whole human being must be kept in focus when examining cells, organs or functional system. For example, if metals interfere with biochemical mechanisms for the production of hormones, the functional and morphological damage caused by hormone imbalance may be remote from the cell affected by the metal. Trace elements are usually considered as those required by man in microgram to milligram quantities. These include iron, zinc, copper, and manganese in larger amounts; selenium, chromium, cobalt, and, possibly, molybdenum are included in lesser amounts. Certain animals on deficient diets have been shown to require tin, nickel and vanadium in order to perform essential functions (WHO, 1973). At present there is no known essential role for lead, cadmium and mercury in biological systems; rather, they may interfere with the role of essential metals by competing for binding sites. (Such metal interactions will be elaborated upon in Chapter 6). Some effects of toxic metals may involve a mechanism in which the metals act as haptens, and protein-bound metals act as antigens, resulting in an allergic type of effect, e.g., metal fume fever and skin sensitization due to nickel and chromium. It is also possible that toxic metals interfere with immunological reactions.

IV.4.2 Effects of cadmium and its compounds

Acute cadmium intoxication usually occurs accidentally after inhalation of cadmium dust or fume in industry and following ingestion of food, water and beverages contaminated with high levels of cadmium. Long-term inhalatory and ingestion exposures may occur via the same respective media in the industrial and general environments and may affect relatively large groups of people. In this section some comments on acute effects will first be given. Later on, more emphasis will be placed upon effects observed in connection with the long-term type of exposure.

IV.4.2.1 Acute effects; general aspects of cadmium toxicity

Acute effects of cadmium have been recognized for a long time and, in man, include pneumonitis resulting from inhalation of high concentrations of cadmium dust or fume. With ingestion of fluids with concentrations of cadmium soluble salts exceeding about 15 mg/liter, vomiting occurs in man. In animals such as rats or mice this reaction does not occur (Schwartze and Alsberg, 1923) and exposure to much higher peroral concentrations is possible. This explains why experimental poisoning by ingestion may be rather easily achieved in rodents, whereas poisoning showing systemic effects requires very long times in man and other primates. Acute effects in experimental animals upon injection of cadmium salts include, e.g., effects on testicles, sensory ganglia, nonovulating ovaries, placenta, and fetuses. Although these latter effects have not been observed in human beings and therefore are of seemingly limited practical relevance, the studies on them have revealed relationships of fundamental interest to metal toxicology, e.g. the interaction between various trace elements, or the influence of tissue binding upon the type of toxic manifestation.

A specific, low molecular weight protein, metallothionein, was detected in equine (Margoshes and Vallee, 1957; Kägi and Vallee, 1960, 1961), and human renal tissue (Pulido et al., 1966). Its role as a sequestering and detoxifying protein for cadmium was subsequently suggested by its isolation from cadmium-exposed animals (Piscator, 1964). The synthesis of metallothionein is inducible by cadmium exposure (Shaikh and Lucis, 1970, 1971; Nordberg et al., 1971b, 1972; Squibb and Cousins, 1974), and such induction may play a telling role in the modification of the toxicological response to cadmium. It was observed that pretreatment with smaller doses of cadmium protected against the development of testicular necrosis (Terhaar et al., 1965; Ito and Sawauchi, 1966; Gunn and Gould, 1970; Nordberg, 1971), lesions in sensory ganglia (Gabbiani et al., 1967a) as well as the lethality of injected cadmium (Gabbiani et al., 1967b; Yoshikawa, 1970). The potential role of metallothionein in the protection against cadmium toxicity has been documented, at least for the testicular effects (Nordberg, 1971, 1972a). The inducible synthesis of metallothionein leads to differences in retention and distribution of cadmium as well as changes in biological effects, and is of basic importance for critical organ concentrations of cadmium and other metals (Nordberg, 1971; Cherian and Vostal, 1974; Vostal, 1975; Nordberg et al., 1975).

Changes in blood pressure have been reported in experimental animals after a single injection of very low doses of cadmium (Dalhamn and Friberg, 1954; Perry et al., 1970). After injection of relatively low doses, morphologically evident tissue damage has taken place in testicles (Parizek, 1957, 1960; Gunn and Gould, 1970; Nordberg, 1971, 1972a), in the nonovulating ovaries of prepubertal rats (Kar et al., 1960) and of adult rats in persistent estrus (Parizek et al., 1968a), and in the placenta (Parizek, 1964). In the last third of pregnancy in the rat, cadmium salts induce a specific and highly lethal syndrome affecting not only fetuses but also mothers (Parizek, 1965); this effect is strictly dependent on the presence of placenta in the maternal organism (Parizek et al., 1969a). Cadmium salts given to pregnant hamsters were shown to be teratogenic (Ferm and Carpenter, 1968). With higher injected doses, changes have been observed in sensory ganglia (Gabbiani, 1966; Gabbiani et al., 1967a). In addition, several visceral organs including the pancreas, kidney, gastrointestinal tract, salivary glands, and spleen display morphological alterations (Kawai et al., 1975). Vascular injury, which has been shown to be involved in the morphogenesis of testicular lesions, may also be the mechanism for the development of the alterations in such organs as the kidney in cases of acute intoxication. The type of lesion seen in, for example, the kidney after acute intoxication differs morphologically from the lesions seen in chronic intoxication (Kawai et al., 1975). Care should therefore be taken in interpreting morphological changes in the parenchymal cells of sensitive organs since these might be evoked, at least partly, by a coexisting circulatory disturbance.

The two categories of renal tissue changes (acute and chronic) have the following morphological features (Kawai et al., 1975). In the acute category, hydropic swelling and acidophilic necrosis prevail; the chronic category is characterized by tubular atrophy. Edema in the surrounding interstitium is conspicuous, and may eventually proceed into interstitial fibrosis and nephrosclerosis at a later stage. The chronic alterations also include mild changes in the walls of blood vessels and marked thickening of the basement membrane of the renal tubules. Acute types of lesions in the tubular epithelium may be seen along with the chronic effect during repeated administration of large doses. The extent and severity of acute epithelial changes correspond roughly to the daily administered dose (review: Kawai et al., 1975).

Nomiyama (1972) and Nomiyama et al. (1973a, 1973b) demonstrated that cadmium can depress vital renal functions, tubular maximum for PAH-transport and tubular reabsorption of low-molecular-weight proteins (myoglobin).

IV.4.2.2 Effects of long-term cadmium exposure

Upon long-term exposure via inhalation, cadmium gives rise to a syndrome including emphysema and effects on the kidney reflected by the appearance of proteinuria (Friberg, 1948, 1950), and increased formation of renal stones (Friberg 1950; Ahlmark et al., 1961; Kazantzis, 1970). Slight to modest anemia also accompanies the clinical picture. Bone manifestations of cadmium poisoning have been observed upon oral exposure in situations in which nutritional conditions have not been optimal or upon inhalation of high concentrations of cadmium in industry (review: Friberg et al., 1974). It is not known with certainty whether this effect can occur in the absence of evident renal effects of cadmium. A further discussion on the mentioned effects will be given below. At low-level long-term inhalation or oral exposures to cadmium, the renal changes are usually the critical effect.

IV.4.2.2.1 Respiratory effects

Friberg (1950) showed that industrial workers exposed to cadmium dust suffered from emphysema with increased residual capacity. These findings have been confirmed by Baader (1951). Similar findings, but also including bronchitis, have been reported (Vorobjeva, 1957; Kazantzis, 1963; Potts, 1965; Adams et al., 1969; Lauwerys et al., 1974) after exposure to cadmium dust. After exposure to cadmium oxide fumes, emphysema (Lane and Campbell, 1954; Bonnell, 1955) and increased residual volume (Buxton, 1956) have been reported.

An association in epidemiological studies between cadmium concentrations in tissues and pulmonary disease has been reported (Lewis et al., 1969; Morgan, 1971; Hirst et al., 1973). This may be explained by the fact that cigarette smoke contains cadmium, and cigarette smoking alone can give rise to chronic respiratory disease (Friberg et al., 1974).

with age-matched controls. However, the emphysematous group contained more heavy smokers than the control group.

Pulmonary changes are inducible in animals upon long-term inhalation of cadmium. Both emphysema and inflammatory changes were reported by Friberg (1950) after exposure of rabbits to cadmium dust. Vorobjeva (1957) found similar changes after intratracheal instillation of cadmium oxide dust in rats. Snider et al. (1973) reported changes resembling human centrilobular emphysema in rats dosed with a 0.1% polydisperse aerosol of cadmium chloride in physiological saline.

IV.4.2.2.2 Renal effects; histopathological and biochemical features

Morphological observations on the nature of the renal damage after long-term exposure to cadmium in human beings have disclosed variable patterns from isolated slight tubular alterations to severe renal lesions. The earliest change seems to be the proximal tubular involvement (Friberg et al., 1974).

In experimental animals, additional morphological changes have been reported. When moderate daily doses of cadmium had been administered, degenerative changes in the proximal tubules were the earliest observable alteration (Axelsson et al., 1968). With prolonged exposure, edema of the interstitium, thickening of the glomerular capsule, and mild or pronounced fibrosis of the basement membrane have also been observed (Bonnell et al., 1960; Axelsson et al., 1968; Stowe et al., 1972).

Pronounced fibrotic and edematous interstitial changes reported in long-term studies, in which relatively high peroral doses of cadmium had been employed, may have been at least partly caused by primary effects on the blood vessels (Fowler and Jones, 1973; Kawai et al., 1975) whereas tubular changes may be elicited by a direct action of cadmium on proximal tubule cells (Kawai et al., 1975). Further aspects of the mechanism behind this latter effect will be given later.

Friberg (1950) described clinically detectable proteinuria in more than 80% of a group of 43 Swedish workers exposed for about 20 years to high air concentrations of cadmium oxide dust. More than 20% of exposed workers also had decreased ability to concentrate the urine. Only slight decrease in glomerular function was observed in several cases. Studies on the character of urinary proteins from these workers (Olhagen, 1950) disclosed that the molecular weight of the main fraction was only 20,000-30,000. In electrophoresis, the albumin fraction was relatively small and an α-globulin predominated in globulin proteins.

Cadmium proteinuria was confirmed by further industrial studies (Friberg et al., 1974) and reports on the increased prevalence of proteinuria in groups from the general population endemically exposed to cadmium were published from several locations in later years (Ishizaki, 1969, 1971; Fukushima Prefecture, 1971; Hyogo Prefecture, 1972; Fukuyama and Kubota, 1972). Total protein in urine as measured by a biuret method showed no significant differences between polluted and nonpolluted areas according to Watanabe et al., 1973, 1974. The precipitating agent was not given in the report.

Molecular weight of urinary proteins in chronic cadmium poisoning varies from 10,000 to 200,000, but more than half of the total protein has a molecular size smaller than albumin, the globulin fraction being represented predominantly by alpha-2, beta-2, and gamma globulins; β_2-microglobulin, retinol binding protein, and gamma globulin L-chains are typical representatives for this group. This type of proteinuria is not specific for cadmium poisoning, being also found in other renal tubular dysfunctions, congenital or acquired (Butler and Flynn, 1958).

Increased urinary excretion of low molecular weight protein has been reported among persons in general populations exposed to cadmium and other metals in the food, both in areas without Itai-itai disease patients (Watanabe and Murayama, 1974) and in areas with high prevalence of Itai-itai disease (Fukushima and Sugita, 1970; Fukuyama and Kubota, 1972). In these studies methods for detection of low-molecular-weight proteinuria were applied primarily in order to evaluate the response to cadmium exposure in population groups.

Since proteinuria is not pathognomonic for cadmium poisoning, proper control groups must be set up.

According to Friberg (1957) and Bonnell et al. (1959) the proteinuria can appear long after cessation of exposure. Piscator (1962b) showed that 24 workers with proteinuria from the group of workers studied by Friberg (1950) retained proteinuria even 10 years after cessation of exposure. On the other hand, Tsuchiya (1974) presented some evidence that proteinuria may be reversible in workers whose exposure to cadmium ceased or diminished considerably.

It has been shown (Friberg, 1952; Axelsson and Piscator, 1966) that urinary cadmium excretion in rabbits is rather low at the beginning of exposure, a sudden increase then occurring concomitantly with the appearance of proteinuria. Nordberg and Piscator (1972) observed a similar phenomenon in mice,and Suzuki (1974) observed it in rats.

Friberg et al. (1974) reviewed the observations obtained in man. Exposed workers without proteinuria excrete small amounts of cadmium, while high excretions up to over 100 µg/24 hours are found in those individuals with proteinuria. Lauwerys et al. (1974), as well as Singerman (1975), have presented results which confirm these findings. Harada, A. (1975) did not find a continuous relationship between exposure level and urinary cadmium among workers with from 6 months to 5 years of exposure; the average cadmium excretion increased suddenly after a certain level of exposure had been achieved. Tubular proteinuria was not found among these workers. In mice with tubular proteinuria, cadmium was found in the urine partly bound to a low-molecular-weight (≈10,000) protein (Nordberg and Piscator, 1972).

Tubular type proteinuria and increased urinary cadmium excretion thus occur relatively early in long-term cadmium exposure. In more advanced cases, multiple tubular defects, such as, e.g. slight glucosuria, may appear before aminoaciduria and phosphaturia develop (Piscator, 1966). Kazantzis et al. (1963) and Adams et al. (1969) found changes in the renal handling of uric acid and calcium. The disturbances in bone mineral metabolism may cause renal stones (Ahlmark et al., 1961; Adams et al., 1969) or osteomalacia (Nicaud et al., 1942).

The significant increase in total excretion of amino acids is considered as a relatively late and unspecific sign. Toyoshima et al. (1973) reported that particularly citrulline and arginine were excreted in large amounts in cadmium-exposed workers. Telling changes in the excretion of these amino acids may occur earlier than changes in the total amino-nitrogen excretion. Patients with Itai-itai disease and other cadmium-exposed persons in the general population have disclosed similar patterns in amino acid excretion. In addition, increased excretion of proline and hydroxyproline has been reported (Sano and Iguchi, 1974). The role of cadmium exposure in inducing proteinuria, glucosuria, and aminoaciduria has been confirmed in long-term exposure through respiratory, parenteral, or peroral administration routes on animals (Friberg, 1950; Axelsson and Piscator, 1966; Nomiyama et al., 1975).

Biochemical mechanisms responsible for renal tubular effects of cadmium have not yet been elucidated. Cadmium may either activate or inhibit a large number of enzymes *in vitro* as well as *in vivo* (Vallee and Ulmer, 1972).

IV.4.2.2.3 Methods for detection of tubular dysfunction in chronic cadmium intoxication; possible indicators of effects

The methodology applied for the detection and evaluation of the tubular proteinuria consists of determining the concentration and electrophoretic distribution of proteins in urine (Butler and Flynn, 1958; Piscator, 1962a, 1962b, 1966). Recent quantitative immunological methods for determination of certain low molecular weight proteins may offer even better possibilities of detecting early changes in urinary excretion of proteins in the future.

The determination of total urinary protein can be accomplished by complete precipitation (as with a reagent containing phosphotungstic acid) and quantitative protein determination with a biuret method (Piscator, 1962b). Complete precipitation is not obtained by trichloracetic acid or sulfosalicylic acid. Another adequate method utilizes complete fixation of urinary protein in a filter paper or cellulose membrane and subsequent staining (Piscator, 1962b).

Electrophoretic separations can be performed in different media, e.g. paper, cellulose acetate, starch gel, agar, agarose, or polyacrylamide. All these methods will give different patterns, and great difficulties obviously arise when patterns obtained by different methods are to be compared.

Among the low molecular weight proteins, ribonuclease and muramidase have earlier been determined in some studies (Piscator, 1966), but β_2 -microglobulin now seems to be the most promising protein since a radioimmunochemical method has been developed (Evrin and Wibell, 1972; Evrin, 1973) which allows its quantitative determination even at its normal levels. This method seems to be the most fruitful for specific determination of an increase in the excretion of a low-molecular-weight protein.

Retinol-binding proteins (RBP) can be quantitatively determined by immunodiffusion techniques (Peterson and Berggård, 1971; Kanai et al., 1971). Peterson and Berggård (1971) and Nomiyama et al. (1973c) demonstrated increased amounts of RBP and β_2-microglobulin in occupationally exposed workers and patients with Itai-itai disease, respectively.

By combining total protein determination, paper electrophoretic separations, and determination of β_2 -microglobulin by the above mentioned method of Evrin, it was possible to detect very small changes in the renal handling of low-molecular weight-proteins by cadmium-exposed workers (Piscator, 1972).

The above mentioned methods are for small-scale studies of selected groups. For large-scale epidemiological studies, which may involve thousands of urine examinations, qualitative methods may by necessity be used. It is of utmost importance in such studies that urine from both controlled and exposed groups are examined by the same person, since a bias may otherwise be introduced. The specific gravity of the urines should also be measured, since different degrees of concentration of the urines may influence the results. In the future, large-scale determinations of β_2 -microglobulin may also be feasible, which would augment the possibilities for detection of increased prevalence of tubular proteinuria in a population.

Increased excretion of these low-molecular-weight proteins (retinol-binding protein, β_2 -microglobulin) may be the most common sign in early stages of cadmium intoxication and their quantitative determination may be an early and specific method of detecting renal tubular impairment in cadmium exposure.

As noted in the previous section (IV.4.2.2.2) glucosuria and aminoaciduria may also appear as a result of the tubular defect. Glucosuria is generally determined by test-tapes, originally intended for finding diabetes. By quantitative determination it is possible to show slight increases in the excretion of glucose, which do not show up with test-tape (Piscator, 1966). Total excretion of amino acids is determined by total amino-nitrogen. Chromatographic determination of specific amino acids may be more sensitive. It should be remembered that the excretion of amino acids and glucose is also dependent on dietary habits.

IV.4.2.3 Other effects of cadmium

It has been stated above that renal tubular impairment is usually the critical effect in long-term, low-level cadmium exposure and that the pulmonary effects are critical in more intensive exposures. However, a number of other effects have also been reported in both human beings and experimental animals as a result of intensive cadmium exposure. The said effects are anemia, hypertension, liver disturbance, and effects on bone tissue, as well as effects on endocrine functions, and genetic, carcinogenic, and teratogenic effects (review: Friberg et al., 1974).

Available data are inconclusive with regard to several of these effects, and more research is needed. Anemia, hypertension, and effects on bone tissue deserve more specific attention here.

IV.4.2.3.1 Anemia

Anemia has been reported both in human beings after industrial exposures to cadmium (Nicaud et al., 1942; Friberg, 1950) and in animals after oral exposures (Wilson et al., 1941). Anemia is usually moderate and includes low haptoglobin levels. This indicates that a hemolytic component is partly responsible for the effect of cadmium. Some animal experiments by Berlin, M. et al. (1961) indicated that an inhibition of iron incorporation into hemoglobin may occur. Other animal experiments have shown decreased availability of iron for the synthesis of hemoglobin in bone marrow. Parenterally administered iron in cadmium-exposed animals prevented the anemia (Friberg, 1955). In the experiments and industrial report in which anemia was induced, it can be postulated to have stemmed from decreased absorption of iron from food. Data are not sufficient to allow a detailed dose-response relationship for anemia in human beings. The occurrence of anemia is probably dependent on the dietary content of vitamin C and iron, since both of these dietary components, if supplied in high amounts, have been shown to prevent anemia in animal experiments (review: Friberg et al., 1974).

IV.4.2.3.2 Hypertension

Animal experiments show that hypertension can be induced by oral and parenteral administration of cadmium. Studies indicating higher body burdens of cadmium in people with cardiovascular disease have come to light (Schroeder, 1967; Friberg et al., 1974). This effect of cadmium has been thought to arise at low concentrations of cadmium in the kidney, at concentrations similar to those found in normal subjects. Systematic studies for hypertension in human beings exposed to excessive amounts of cadmium in the general environment or in industry have not been reported. (See also section IV.6).

IV.4.2.3.3 Effects on bone tissue

After the Itai-itai disease (osteomalacia) in Japan had been shown to be associated with cadmium exposure, interest in effects of cadmium on osseous tissue was stimulated. The effect of cadmium on bone can be secondary to an effect on the renal tubules, since the kidneys have key functions in the regulation of the calcium and phosphorus balance in the body. Osteomalacia and severe osteoporosis have been reported among cadmium exposed workers (Nicaud et al., 1942; Gervais and Delpech, 1963; Adams et al., 1969; Kazantzis, 1975) as well as among persons in general populations with high cadmium exposure and probable calcium deficiency (Itai-itai disease, see Friberg et al., 1974).

Kobayashi (1973) reported that cadmium exposure caused a negative calcium balance in rats. A decreased calcium absorption and histopathological changes in the duodenum were observed in rats receiving 50 μg Cd/g drinking water (Sugawara and Sugawara, 1974). Kawai et al. (1975) described decalcification and cortical atrophy in the skeletal tissue during long-term peroral exposure of experimental animals. These alterations were observed earlier than the morphological changes in the renal tissue.

Feldman and Cousins (1973) and Kimura et al. (1974) demonstrated that cadmium may decrease the production of the active vitamin-D metabolite, 1,25-OH-cholecalciferol, in vivo.

Osteomalacia can be induced by a combination of cadmium exposure and calcium deficiency, but the mechanism is still unclear (Itokawa et al., 1974). Since nutritional factors such as vitamin D and calcium are highly influential, the effect on the skeletal tissue may possibly be a critical one in cadmium exposed populations for whom intakes of these constituents are marginal.

With regard to the etiology of Itai-itai disease, it was defined as renal osteomalacia, for which the main etiological factor was cadmium, but host factors such as malnutrition, pregnancy and aging, are of large consequence. However, Kajikawa et al. (1973) reviewed 11 autopsies of Itai-itai patients and came forth with a speculative statement that Itai-itai disease is a nutritional osteomalacia caused by the insufficient intake of vitamin D, as well as other malnutritional factors. Kajikawa et al. (1973) referred to studies from 1906 showing that osteomalacia and rickets had been prevalent in Toyama Prefecture as early as that time. These authors used this as supporting evidence for their hypothesis.

IV.4.2.4 Critical effects and critical organs at exposure to cadmium and its compounds

In long-term exposures to cadmium, the kidney appears to be the critical organ and the most commonly studied critical effect is manifestation of proteinuria. Increased urinary excretion of low molecular weight proteins, such as RBP and β_2-microglobulin, may be considered as an early and specific renal effect of cadmium. Fully developed tubular proteinuria, characterized by the prevailing presence of proteins with molecular weight up to that of plasma albumin and higher, is a typical sign of chronic cadmium poisoning; this type of proteinuria is, however, not specific for cadmium effects, having been found in other renal tubular dysfunctions, congenital or acquired. The same conclusion regarding nonspecificity can be applied to glucosuria and aminoaciduria, frequently reported in chronic cadmium poisoning. Bone manifestations, such as osteomalacia, can be induced by experimental cadmium exposures or may appear in population groups with high exposures and non-optimal nutritional conditions; mechanisms of their origin may, however, be secondary to the effects of cadmium on renal tubules and subsequent changes in calcium and phosphorus balance.

After inhalation of high concentrations of cadmium fumes or dusts, the respiratory system becomes the critical organ and acute pneumonitis or chronic emphysema with increased residual capacity and bronchitis represent critical effects of cadmium, particularly in exposures such as may occur in industry.

Acute effects of injected cadmium on testicles, sensory ganglia, nonovulating ovaries, placenta and fetuses were demonstrated in experimental animals but have not been observed in human exposures so far.

IV.4.3 Effects of lead and its compounds

Lead effects may occur in a number of organs and systems and may follow exposure to either inorganic or organic compounds of lead. The comments in this document relate principally to inorganic lead exposure except for one section dealing with consequences of exposure to organic forms (tetraethyl lead). The major effects of lead concern three systems, namely, hematological, neurological and renal. Effects on other organs are also discussed briefly, but are not considered for establishing dose-response relationships.

IV.4.3.1 Effects of inorganic lead compounds

IV.4.3.1.1 Hematological effects

Lead exposure may give rise to microcytic anemia, shown by biochemical methods to be caused by a combination of inhibition of hemoglobin synthesis and shortened life span of circulating erythrocytes. From the biochemical point of view, the most impressive effects of lead are on the biosynthesis of hemoglobin. According to numerous studies, it may be concluded that lead can affect many steps in the pathway of heme synthesis (Griggs, 1964). Since several recent reviews give detailed accounts of this matter (Waldron, 1966; Airborne Lead in Perspective, 1972; Goyer and Rhyne, 1973), only a brief recapitulation will be given here. *In vitro* experiments using avian erythrocytes have been particularly fruitful because these cells contain the complete heme biosynthetic system. Such studies show that aminolevulinic acid dehydrase (ALA-D), catalyzing the formation of porphobilinogen (PBG) from ALA, and heme synthetase (heme-S), incorporating iron into protoporphyrin IX (PP IX), are the enzymes most sensitive to the action of lead (Eriksen, 1952; Dresel and Falk, 1956a, 1956b). In addition it has been demonstrated by *in vitro* experiments that high lead concentrations inhibit ALA-synthetase (ALA-S), catalyzing the formation of ALA from glycine and succinate, and coproporphyrinogen decarboxylase (CPG decarboxylase), converting CPG into PP (Goldberg et al., 1956; Kreimer-Birnbaum and Grinstein, 1965; Wada et al., 1972; Wada, 1975).

Recent *in vivo* studies on heme biosynthesis in erythroid cells of bone marrow from workers exposed to lead show a remarkable reduction of the activities of ALA-D and heme-S, a slight elevation of ALA-S activity, and a parallel reduction of ^{14}C-glycine incorporation into heme and globin (Wada et al., 1972; Wada, 1975). Increased activity of ALA-S, in contrast to the results of the *in vitro* study by Goldberg et al. (1956), is not likely to imply the activation of the enzyme by lead but may represent a positive feedback mechanism in response to the decreased heme content in erythroid cells which has resulted from the inhibited heme biosynthesis by lead.

Depression of the activity of ALA-D in circulating erythrocytes occurs without fail in lead poisoning (Lichtman and Feldman, 1963; Bonsignore et al., 1965; Hernberg and Nikkanen, 1972). Partial inhibition has been demonstrated at lead levels far below those commonly thought to be toxic (De Bruin and Hoolboom, 1967; De Bruin, 1968; Hernberg et al., 1970; Hernberg and Nikkanen, 1970; Miller et al., 1970; Weissberg et al., 1971; Hernberg and Nikkanen, 1972; Tola et al., 1973; Wada et al., 1973; Sakurai et al., 1974; Singerman, 1975). In fact, the inhibition of erythrocyte ALA-D activity is the most sensitive effect known for the action of lead.

The blocking effects of lead upon various steps in the heme synthesis are manifested as increased excretion of ALA-U, CP-U, elevation of ALA in serum, and increased free erythrocyte protoporphyrin IX (FEP). These abnormalities can be attributed to the combined effect of the inhibition of heme biosynthetic enzymes other than ALA-S, and a positive feedback mechanism upon certain steps, induced by reduced heme content in the heme forming cells.

It can be concluded that the combination of (1) inhibition of ALA-D activity in circulating erythrocytes, (2) increased excretion of ALA-U and (3) increased excretion of type III

CP-U, and (4) accumulation of FEP, is quite pathognomonic for lead; thus, lead poisoning can be distinguished from all other disorders by keeping this in mind.

Shortening the life span of circulating erythrocytes is the other main mechanism by which lead causes anemia. In chronic poisoning the shortening is moderate, and thus slighter than in most hemolytic anemias (Rubino, 1962; Sroczynski et al., 1965; Hernberg et al., 1967). According to several studies, the survival time is rarely reduced below 60 days. Using *in vivo* labelling with ^{3}H-di-isopropylfluorophosphonate, it has been possible to show slight shortening (to about 100 days) of the erythrocyte life span in a group of lead workers with blood lead levels between 59 and 162 µg/100 ml (Hernberg et al., 1967).

The mechanism by which lead shortens the erythrocyte life span is not well understood (Griggs, 1964; Waldron, 1966; Harris and Elsea, 1967). It might be that the microcytic anemia i.e. the size and shape of the red blood cells explains the increased vulnerability. A number of observations show that the osmotic resistance of erythrocytes from patients with lead poisoning is increased (Griggs, 1964; Waldron, 1966; Harris and Elsea, 1967). Increased mechanical fragility has also been reported. However, studies dealing with these phenomena are equivocal (Hasan and Hernberg, 1966; Waldron, 1966). The biochemical changes at the erythrocyte membranes responsible for changes in the mechanical fragility and the osmotic resistance are not well known. Loss of potassium and water occurred when lead salts were added to erythrocytes *in vitro* (Passow et al., 1961), and when whole blood from lead workers was incubated at 37°, a potassium loss occurred as well (Hasan et al., 1967a). This phenomenon may or may not be related to the fact that erythrocyte membrane Na^+/K^+-ATPase activity is inhibited by lead (Hasan et al., 1967b; Hernberg et al., 1967). Red cell glutathione has also been shown to be diminished due to the action of lead (Roels et al., 1974). The phenomena recapitulated above represent only a few possible explanations for the lead-induced shortening of red blood cell life span.

A consequence of all these effects of lead on hematopoiesis is that the production of red blood cells increases, which is manifested by reticulocytosis. Basophilic stippling of the erythrocytes is another feature of lead-induced anemia, and has earlier been used for the diagnosis of lead poisoning. However, this nonspecific phenomenon correlates poorly -- if at all -- with the intensity of lead poisoning.

IV.4.3.1.2 Neurological effects

Lead exposure may have serious effects on the central and peripheral nervous systems. Central nervous system effects are most frequent in childhood, from pica or other forms of childhood lead exposure. Peripheral nervous system effects are more characteristic of long-term lead exposure in adults. However, children may also manifest a peripheral neuropathy and adults may have central nervous effects particularly if exposure is short-term and very heavy, e.g. paint chippers in missile silos (Lambie, 1967). Tetraethyl lead exposure in the adult may be associated with central nervous system effects.

IV.4.3.1.2.1 Central nervous system effects (encephalopathy)

The classical signs and symptoms of lead encephalopathy are ataxia, coma and convulsions. Acute encephalopathy may be fatal. Survivors of lead encephalopathy may sustain residual brain damage which is manifested by mental and/or neurological impairment.

The most prominent morphological changes noted in the brain are cerebral edema, proliferation and swelling of endothelial cells accompanied by dilatation of capillaries and arterioles, proliferation of glial cells and focal necrosis, and neural degeneration. Another common feature of lead encephalopathy is a diffuse astrocytic proliferation in the grey and white matter. The extent of neuronal loss in cortical grey matter is variable, but provides a morphological basis for the permanent sequelae of lead encephalopathy (Pentschew and Garro, 1966).

From the few autopsied cases of lead poisoning in which brain tissue has been analyzed, it has been found that lead concentrations in this organ are not as high as in other organs such as liver and kidney. In one case in which lead was measured in more than one part of the brain, the highest concentrations were found in the cortical grey matter and basal ganglia (Klein et al., 1970). A more detailed mapping of variation in lead concentration in different parts of the brain of lead poisoned dogs showed similar results (Stowe et al., 1972). These studies support a relationship between areas of the most marked histological change and high lead concentrations.

The effects of lead on the central nervous system may not be completely reversible (Chisolm and Harrison, 1956; Byers, 1959). Sequelae include mental retardation, seizures, paralysis and optic atrophy (Perlstein and Attala, 1966). Likelihood of sequelae is related in some degree to severity of symptoms prior to treatment. The risk of permanent neurological complications increases with repeated episodes of acute intoxication or with chronic excessive exposure to lead (Chisolm and Harrison, 1956; Byers, 1959).

Whether less severe or subclinical effects of lead on the central nervous system are recognizable in children is a topic of current debate. A relationship has been suggested between slight loss of intelligence (de la Burde and Choate, 1972) and hyperactivity (David et al., 1972) on the basis of studies carried out primarily in school age children with epidemiological and clinical histories suggestive of increased lead absorption during the preschool years. Behavioral and psychological changes and loss of fine motor function (Pueschel et al., 1972; Albert et al., 1974) have been reported in young children with blood lead levels $\gtrsim$ 50-60 μg Pb/100 ml blood during the preschool years.

At the present time, only partial experimental confirmation of such lead effects is on hand. Silbergeld and Goldberg (1974) have induced hyperactivity in mice exposed to lead through their mother's milk, and, like humans with hyperactivity, the lead treated mice responded paradoxically to the central nervous system stimulants. Also, Carson and coworkers (1974) have shown a slowness in learning in lambs prenatally exposed *in utero* to maternal blood lead levels of 34 μg/100 ml during gestation.

IV.4.3.1.2.2 Peripheral nervous system manifestations

Peripheral neuropathy is seen only sporadically nowadays and almost exclusively under conditions of uncontrolled exposure. The neuropathy is characterized by selective involvement of motor neurons with little sensory involvement. Mild forms exist too, which can be assessed by electrophysiological techniques (Catton et al., 1970). Experiments with isolated perfused ganglia (Kostial and Vouk, 1957) or isolated sciatic nerves (Manalis and Cooper, 1973) have shown that a reduction of the end-plate potential by a presynaptic block may be the mechanism of the lead effect on nerves. Pathological changes in experimental lead-induced peripheral neuropathy include segmental degeneration of myelin sheaths and axonal degeneration (Schlaepfer, 1969). (See also section IV.5.3.2.2.2).

IV.4.3.1.3 Renal effects

In children with short-term but heavy exposure to lead, functional and morphological changes occur in proximal renal tubular cells (Marsden and Wilson, 1955; Chisolm and Leahy, 1962). The functional effects include the Fanconi syndrome manifested by aminoaciduria, glucosuria, and hyperphosphaturia in the presence of hypophosphatemia.

Morphologically there are nonspecific degenerative changes in proximal tubular lining cells, including mitochondrial swelling, and a more specific change, eosinophilic, dense staining nuclear inclusion bodies. Electron microscopy of the inclusion bodies shows that they have a characteristic outer fibrillar margin useful for distinguishing them from other nonspecific bodies. A number of experimental studies using autoradiography, direct chemical analysis, and X-ray microanalysis show that the bodies are a lead-protein complex and may have a role in the excretion of lead. A recent study shows that the bodies are excreted during EDTA chelation and may represent one source of EDTA mobilizable lead (Goyer et al., 1974). The functional and morphological renal effects of relatively short-term lead exposure (probably less than 1 to 5 years) are reversible following treatment with chelating agents (Chisolm and Leahy, 1962).

The effect of long-term exposure to lead on the kidney differs from the short-term effects just described and is seen in adults with heavy occupational or other exposures to lead. It consists of a nonspecific nephropathy characterized morphologically by intense interstitial fibrosis, tubular atrophy and dilatation with relatively late involvement of glomeruli. Tubular dysfunction is manifested by a defect in uric acid excretion often resulting in hyperuricemia, and sometimes in gout. The typical Fanconi syndrome has not been documented in the adult form of lead nephropathy. Reduced glomerular filtration is a late effect of lead nephropathy but renal failure may occur. There have been many studies of lead nephropathy in occupationally exposed individuals from different parts of the world. These have been reviewed and their associated morphological changes summarized (Goyer, 1971).

A weighty question is whether or not children with short-term exposure to lead are more susceptible to a form of chronic nephropathy later in life. Ten to twenty year follow-up studies of children in the United States known to have had lead poisoning in childhood showed no increase in nephropathy in later life (Tepper, 1963; Chisolm, 1970). However, studies from Australia (Henderson, 1954; Emmerson, 1968) suggested that long-term exposures to lead in childhood or adolescence may progress to a chronic nephropathy with the same characteristics as in chronic lead nephropathy in occupational exposure, that is, a high incidence of hyperuricemia and severe interstitial fibrosis. It may be that decisive differences existed between the form of the lead nephropathy seen in Australia and that seen in young people in the U.S.A., perhaps with regard to age and length of exposure to lead.

IV.4.3.1.4 Gastrointestinal effects

The gastrointestinal system is invariably involved in obvious lead poisoning, and symptoms from the alimentary tract occur frequently even in milder forms. Acute lead colic may result from massive short exposure to lead, but it may also develop quite suddenly in subjects with long-term exposure. It is characterized by attacks of crampy diffuse abdominal pain, generalized muscle aches and constipation. The skin of the patient is pallid and the blood pressure may be elevated. Lead lolic may be preceded by more diffuse symptoms. Prolonged, severe constipation is considered a warning of incipient colic. The mechanism for the gastrointestinal effects caused by lead is unknown (Airborne Lead in Perspective, 1972).

IV.4.3.1.5 Teratogenic and other reproductive effects

Although effects of lead on reproductive fitness have been suspected to occur in individuals with increased body burden of lead, this effect has received limited study in man. From experimental studies, lead has been found to be toxic to gametes of both sexes, resulting in reduced numbers and size of offspring from lead-poisoned rats (Stowe and Goyer, 1971). These studies imply that lead is transported via sperm or ova to the zygote, but actual transport of lead in this manner has not been demonstrated. Histopathological changes in ovaries in lead-poisoned Rhesus monkeys have been demonstrated (Vermande-van Eck and Meigs, 1960), but such changes have not been seen in man.

Reports of sterility, stillbirths, and miscarriages among women working in the lead industry have been summarized in the older literature (Oliver, 1914; Lund, 1936; Cantarow and Trumper, 1944). There are no recent data on occupationally exposed women in the childbearing age range concerning possible effects of increased body burden of lead on reproductive fitness or on the fetus. Likewise, there are few experimental studies demonstrating a possible teratogenic effect of lead. Ferm and Carpenter (1967) showed that the intravenous administration of lead salts to the golden hamster at a specific time during gestation results in skeletal malformations. Of greater concern, particularly with regard to occupational low-level lead exposure of women in the childbearing age range, is the risk of central nervous system effects in the fetuses.

IV.4.3.1.6 Endocrine effects

An effect of lead on various endocrine glands has been reported. The observations have been made primarily in individuals who had consumed "moonshine" whiskey for many years (Sandstead, 1973). Functional tests of the pituitary-adrenal axis (Sandstead, 1973), the pituitary-thyroid axis (Sandstead et al., 1969), the pituitary-gonadal axis (Sandstead, 1973) and the renin-aldosterone system (Sandstead et al., 1970; McAllister et al., 1970) have been found abnormal. The impairment of ^{131}I uptake by the thyroid has been confirmed in rats chronically intoxicated with lead (Sandstead, 1967). The impairment of the renin-aldosterone response to sodium deprivation has been reversed by treatment with $Ca\text{-}Na_2\text{-}EDTA$ (McAllister et al., 1970). Therefore it seems that these latter two adverse effects are indeed due to lead. It is possible that other undefined substances in the "moonshine" contributed to some of the abnormalities. The practical importance of these findings is at present unknown.

Others have also reported an adverse effect of lead on the pituitary-gonadal axis (Raule and Morra, 1952) and other organs of the endocrine system (Pines, 1965; Muraslov, 1966). These reports are generally supportive of the above interpretation of the studies cited above.

IV.4.3.2 Effect of alkyl lead compounds

Alkyl lead compounds, in contrast to inorganic lead, are readily absorbed by inhalation. This may be followed by central nervous system effects and may occur without hematological, gastrointestinal, or renal effects. Concentrations of lead in the brain of persons dying from tetraethyl lead intoxication are much higher than those in the same organ of persons with inorganic lead poisoning (Cassells and Dodds, 1946; Cummings, 1967). Other soft tissues and bone may contain less lead than is found in persons with inorganic lead toxicity but this depends on length of exposure. Alkyl lead compounds are lipid soluble which is thought to explain the tissue distribution. The lipid soluble tetraethyl lead, though it might interfere with membranes, is not responsible for the toxic effect at the biochemical level. The toxic metabolite is triethyl lead which is formed mainly in the liver (Cremer, 1959). Although there are some data on poisoning by tetraethyl lead compounds, they were considered too scanty to establish dose-effect or dose-reponse relationships.

IV.4.3.3 Critical effects and critical organs at exposure to inorganic and organic lead compounds

The critical effect of lead is adverse interference with heme synthesis. Increased concentrations of heme intermediates in blood or urine (ALA-S, ALA-U, CP-U, FEP) are evidence of critical effects of lead on heme synthesis. Decreased ALA-D activity in red blood cells is regarded as a subcritical effect.

According to the knowledge at hand, the critical organ for lead effect is the hematopoietic bone marrow. Continuing research may develop methods for measuring neurochemical or neurophysiological disturbances which underlie nervous system manifestations occurring prior to the known heme synthesis effects. It is difficult now to state whether or not nervous disorders may precede hematological disturbances in particular circumstances.

For tetraethyl lead exposure, particularly following short-term inhalation, the critical organ is the central nervous system.

IV.4.4 Effects of mercury and its compounds

It is evident that the biological effects of mercury and its compounds are determined by the different physical and chemical forms of mercury. These have been referred to in an earlier section. In some parts of the following review, representing more general knowledge on mercury toxicology, references have not been given. Recent reviews where such references may be found are ICMACMC (1969), Friberg and Vostal (1972) and Clarkson (1972a, 1972b).

IV.4.4.1 General biochemical aspects of the toxicity of mercury and its compounds

The action of mercury and its compounds at the biochemical level is poorly understood. Biochemical studies and mechanisms of action are complicated by the fact that mercury cations such as Hg^{++}, $C_6H_5Hg^+$ and CH_3Hg^+ are nonspecific enzyme inhibitors. Mercuric cation possesses a high chemical affinity for -SH groups (Rothstein, 1973). Since the -SH group is found in most protein molecules, mercury cations can inhibit most enzymes with which they come into contact in sufficient concentrations. Webb (1966) has listed hundreds of enzymes inhibited by mercury *in vitro*. The -SH group is also essential to the integrity of cell membranes and mercury has been demonstrated to disrupt most membrane functions (active transport, permeability properties) *in vitro* (Rothstein, 1973).

Despite the lack of specificity in biochemical systems *in vitro*, mercury and its compounds nevertheless exhibit remarkable selectivity throughout the whole organism. Many studies have been reported testing the hypothesis that the selective distribution in the body may be responsible for selective toxicity (Berlin, M., 1963; Clarkson, 1972a, 1972b). Recently, investigations have been made with the various complexes and chelates (biocomplexes) formed by mercuric cations *in vivo* in various tissues and body fluids in order to see if this information might help in understanding the selective distribution and toxicity. No general conclusion can be made from the results obtained to date, but certain findings are worth noting. Norseth (1968) reported that Hg^{++} accumulates selectively in lysosomes and suggested that cellular toxicity may be produced by the release of hydrolytic enzymes. That mercury is taken up by lysosomes has also been demonstrated morphologically (Fowler, 1972; Fowler et al., 1974). Alterations in lysosomal and microsomal enzymes have been discovered in renal tissue of animals that accumulated Hg^{++} as well as $MeHg^{+}$ in renal tissue upon $MeHg^{+}$ exposure (Fowler et al., 1975).

Biochemical studies on the binding of Hg^{++} in tissues have provided some insight into renal toxicity and into the development of kidney tolerance. Observations by Kessler et al. (1957), Weiner et al. (1962), Miller and Farah (1962), and Stoytchev et al. (1969) indicate that Hg^{++} forms a two-point attachment to a receptor (active site) in kidney tissue. One point of attachment is a thiol group and the other is a nonthiol ligand.

Clarkson and Magos (1966) identified three classes of binding sites in kidney tissue distinguished by the affinity for Hg^{++}. Kidney levels of Hg^{++} as observed in not excessively exposed human beings (Kägi and Vallee, 1960, 1961) correspond to saturation of the highest affinity class of sites. Subsequently Wisniewska et al. (1970) demonstrated that Hg^{++} may attach to metallothionein in kidney cells under conditions of experimental exposure of rats to Hg^{++}. The levels of a metallothionein-like protein, as measured by Piotrowski et al., 1973) corresponded roughly to the tight binding sites described by Clarkson and Magos (1966).

It has been claimed that metallothionein plays an important and protective role, and that once the binding capacity of this protein has been saturated, toxic effects appear in the kidney. This organ is known to be able to accumulate much higher levels of mercury during chronic exposure than the toxic level observed after a single dose, without any detectable deleterious effect. Mercury is able to induce the synthesis of a metallothionein-like protein in kidney tissue (Wisniewska et al., 1970; Piotrowski et al, 1973; Nordberg, M. et al., 1974) but not in liver. Whereas kidney levels of mercury rise on repeated dosing, the liver levels are stable (Piotrowski et al., 1973, 1974).

The biochemical lesion underlying $MeHg^{+}$ toxicity has not yet been elucidated. Yoshino et al. (1966) and Cavanagh et al. (1970) studied animals given high single doses of methylmercuric compounds. They claimed that the decrease of protein synthesis was the first detectable change in cell function and was accompanied by morphological abnormalities in the ribosomes. Bull (1974) reported interference with enzymes of the respiratory chain in the CNS of rats exposed to 0.05 mg per kg daily for 14 days. The relevance of these findings to poisonings due to long-term exposures in man has not yet been established.

The kidney toxicity of phenylmercuric compounds as reported in animal experiments (Fitzhugh et al., 1950) is probably due to rapid biotransformation to inorganic mercury (review: Clarkson, 1972a, 1972b).

IV.4.4.2 Effects of elemental mercury

Short-term exposure to high concentrations of mercury vapor may give rise to symptoms of pulmonary irritation, and central nervous system involvement. Occupational exposure to mercury vapor is usually long-term. Sometimes there is a combination of exposure to vapor and dust of inorganic and/or organic salts of mercury. In long-term exposure to mercury vapor at higher concentrations, CNS symptoms and signs are most commonly found, with tremor and psychological disturbances (erethismus mercurialis) being most prominent. Proteinuria may also occur, possibly followed by the nephrotic syndrome in rare cases, but definitive evidence concerning the latter is not available in humans.

Gingivitis, stomatitis and excessive salivation may occur in mercury-exposed workers, who may sometimes develop dermatitis as well. Mercurialentis (a discoloration of the anterior lens capsule) is seen following long-term exposure to mercury, but does not indicate intoxication (ICMACMC, 1969). Anorexia, weight loss, anemia, and muscular weakness have been reported. Smith et al. (1970) showed that loss of appetite and weight correlated well with exposure.

A syndrome that is characterized by decreased productivity, increased fatigue, loss of self-confidence, muscular weakness, vivid dreams, depression etc., has been subject to much discussion in the literature from USSR. This syndrome has been called the asthenic-vegetative syndrome or, when connected with mercury exposure, micromercurialism (Trachtenberg, 1969; review: Friberg and Nordberg, 1972).

Singerman and Catalina (1969) demonstrated inhibition of red cell membrane Na^+K^+ ATP-ase in industrial workers exposed to mercury vapor. They also reported an increase in the total activity of serum and urinary lactic dehydrogenase and an increase in the cathodic molecular fractions (isoenzymes) No. 4 and No. 5. No significant changes were found in other enzymes studied which included transaminases and cholinesterase.

IV.4.4.3 Effects of inorganic mercury compounds

Single and short-term exposure to high doses of inorganic mercury is now a relatively rare occurrence, but can be found in accidental or suicidal intake or during occupational exposure. The clinical manifestations of oral intake of mercuric salts include gastroenteritis with abdominal pain, nausea, vomiting, bloody diarrhea, and severe renal disorder leading to anuria and uremia.

The effects of easily soluble mercuric salts upon the kidney have been confirmed in a number of animal experiments (review: Skerfving and Vostal, 1972). Acute total or segmental necrosis of the terminal portions of the renal tubules has been reported in rabbits, guinea pigs, and frogs after injection of mercuric chloride. At higher doses the initial and middle portions of the proximal tubules are also affected. Electron microscopical changes in the mitochondria of the proximal tubules in the rat kidney have been reported after repeated subcutaneous administration (Bergstrand et al., 1959). At injection of high doses of mercuric chloride, on the order of 10 mg/kg or higher, there is an evident ischemia in the renal cortex.

Disturbances of blood flow within the cortical tissue are thought to be of importance for elicitation of the tubular changes at these high doses. It has been postulated that a primary decrease in glomerular filtration rate due to afferent arteriolar constriction takes place at such doses. At doses lower than 5 mg of mercury/kg body weight, vascular disturbance does not complicate the picture according to Biber et al. (1968), who did find necrosis of tubular epithelium in the distal three-fourths of the length of the proximal tubules and no anuria.

Concerning long-term exposures, there have not been many reports on morphological changes in the tissues of patients or experimental animals. However, Kazantzis et al. (1962) performed renal biopsy in two cases of workers exposed to mercury vapor and inorganic mercury salts. The workers had high urinary losses of protein, hyaline casts in urinary sediment and normal glomerular filtration rates. Renal biopsy showed abundance of lipids and vacuolization in the epithelium of the proximal tubules. No abnormalities were seen in the glomeruli. Renal concentrations of mercury ranged between 10 and 20 µg/g fresh tissue.

IV.4.4.4 Effects of organomercury compounds

Organomercurials fall into classes with different stability in biological systems, as mentioned earlier in this report. A subdivision of organomercurials into more stable compounds like alkylmercury salts and less stable compounds like phenyl- and alkoxylalkylmercury compounds is necessary. It is also evident that the symptomatology and the type of biological effects differ between these two types of compounds.

IV.4.4.4.1 Methylmercuric compounds

Symptoms observed in man after exposure to methylmercuric compounds are dominated by neurological disturbances. Symptoms of methylmercury poisoning may occur weeks to months after an acute exposure to toxic concentrations (ICMACMC, 1969). The symptoms and signs of poisoning after short- and long-term exposure include numbness and tingling of the lips, mouth, hands and feet, dysarthria, ataxia, concentric constriction of the visual fields, blurred vision, blindness, deafness and an impaired level of consciousness. With severe intoxication the symptoms and signs were partially reversible in the relatively short-term exposure in Iraq, in contrast to irreversibility after much longer exposure in Minamata. Infants who were exposed prenatally to large amounts of $MeHg^+$ had signs of cerebral palsy, convulsions and in some cases, blindness (Clarkson and Marsh, 1975).

Morphological findings in the brain in fatal cases of methylmercury poisoning include severe changes in the cerebral cortex, especially of the visual cortex, including degeneration of nerve cells, and changes in the granular layer of the cerebellum. All or some of these sites may be involved (Hunter and Russell, 1954; Takeuchi, 1968; Nordberg et al., 1971a; Grant, 1973). Most of these morphological changes have been recorded in both human beings and in animals, e.g. cats and monkeys. There are marked species differences, especially when comparing the metabolism of $MeHg^+$ in rodents to that in primates. Observations on the CNS effects of $MeHg^+$ in rats therefore cannot be applied to humans.

The alterations in the nerve cells seen in the microscope after experimental $MeHg^+$ intoxication have had a topographical distribution which has corresponded to the topographical localization of subcortical accumulation of mercury. A direct pathogenic relationship between this accumulation and the elicitation of neuronal damage thus seems likely. It is not yet clear how this mechanism comes about, but it may be that a primary lesion occurs in the function of the glia cells which secondarily gives rise to the neuronal necrosis (Nordberg et al., 1971a; Grant, 1973). This mechanism may also be related to the latency time clinically observed in $MeHg^+$ intoxication.

$MeHg^+$ also causes brain damage to the human fetus as was initially observed in the Minamata area of Japan in 22 infants who were born between 1953 and 1959. They had clinical features of cerebral palsy. A few of the mothers experienced paresthesias during pregnancy but most of them did not have other features of Minamata disease. One of those 22 infants had a retinal anomaly and another had malformation of an external ear (Harada, Y., 1968).

Apart from cerebral palsy, the existence of teratogenic effects is not clear. Data on the relationship between the maternal and fetal toxicity of $MeHg^+$ have been given by Clarkson and Marsh (1975). They concluded that fetal toxicity occurs at approximately the same exposure levels that cause early maternal toxicity. It may be that early effects of $MeHg^+$ can result from the impact of $MeHg^+$ on cell division and chromosome segregation. Indeed, chromosomal alterations have been witnessed (Skerfving et al., 1970; Ramel, 1972; Skerfving et al., 1974). Litter size was reduced in untreated female rats allowed to mate with male rats treated with methylmercuric compounds. These results have been interpreted as evidence for a dominant lethal mutation (Khera, 1973).

IV.4.4.4.2 Ethylmercuric compounds

Most clinical data about poisoning by ethylmercuric compounds are derived from patients who had consumed seed grain that had been treated with ethylmercury preparations. It is probable, however, that other mercurials were partially responsible for the symptoms and signs that arose. The neurological features were less marked than in $MeHg^+$ poisoning, but gastrointestinal and renal effects were more prominent (Jalili and Abbasi, 1961; Damluji, 1964).

IV.4.4.4.3 Phenyl- and alkoxyalkylmercuric compounds

Methoxyethylmercuric compounds have come to replace methylmercuric ones as fungicides after the recognition of the latter compounds' toxicity. Methoxyethylmercuric salts have only given rise to a few reported cases of poisoning. The symptoms have been loss of appetite, diarrhea, weight loss, and fatigue. The symptoms probably are ascribable to the action of inorganic mercury, resulting from the degradation of the methoxyethylmercuric radical in the body. Kidney damage with albuminuria and occasionally a nephrotic syndrome also have been reported after exposure to methoxyethylmercuric compounds. (For references see ICMACMC, 1969).

In spite of the rather extensive use to which phenylmercuric compounds have been subject, very few cases of intoxication have been reported. An irritant effect on the skin has been observed, but systemic effects are rare. In an acute case in a worker reported by Goldwater et al. (1964) mild evidence of kidney damage occurred. Long-term exposure to phenylmercuric salts has not given any conclusive evidence of toxic effects in industrial exposure situations.

IV.4.4.5 Summary and definition of critical organs in mercury exposure

The critical organ in exposure to mercury and mercurials varies with the type of compound, with dose, with route of absorption, with duration of exposure, and with stage of development of the organism.

After severe short-term exposure to mercury vapor, the critical organ is the lung. With several repeated exposures, the critical organ is likely to be the brain, and after prolonged exposure the critical organ is the brain.

After exposure to mercuric salts, whether absorbed by ingestion or by inhalation, the proximal tubule of the kidney is the critical organ.

The critical organ after exposure to ethyl- and methylmercuric compounds is the brain in man. In pregnant women, the fetal brain may be regarded as the critical organ. For phenylmercuric and methoxyethylmercury compounds, the critical organ is likely to be the kidney.

IV.5 DOSE-RESPONSE AND DOSE-EFFECT RELATIONSHIPS

IV.5.1 General aspects

The concept of dose-response relationship is central in toxicology. In its simplest form it is usually displayed graphically as an S-shaped curve with dose on the x-axis and response on the y-axis. It can be visualized that a complete graphical summarization of the relationship between dose and response, if different effects are taken into consideration, would require at least a family of curves and a three-dimensional model.

There will be no attempt to reduce the complexities of the dose-response relationships and dose-effect relationships to a single model, but rather to point out some of these complexities. The limitations as well as the utility of simple expressions may then be better understood and communicated.

The distinction between effect and response has been elaborated upon in earlier sections. Effects are usually quantifiable and graded (as to degree), so that with, for example, the urinary excretion of ALA (an effect) after lead exposure a dose of one unit (here arbitrarily chosen) may produce no measurable increase in excretion. A dose of 2 units may produce an increase of y above the background levels, and a dose of 3 units may produce another defined increase in excretion of ALA.

Death, as a biological effect, is not usually considered to be graded, but instead an all-or-none-condition. Such an effect is called a quantal effect. To deal with these, one takes a population given a specific dose and measures the percentage showing this specific effect; this percentage value has been defined previously as the response. Using this definition, an LD_{50} value may be described as the dose expected to cause a 50% response among the population tested for the lethal effect.

The distinction between "all-or-none" and "graded" effects is not an absolute one. In a physiological sense, individual fibers or cell components may function only on an all-or-none basis, but when a very large number of these units react to a chemical together, the summation of their individual actions may yield measurements that one observes as graded effects. Many graded effects can be expressed as a percentage of the maximum effect observable.

The important distinction is not dependent upon the observation being made in percentage units. The important distinction is that response designates the enumeration of "reactors" or "non-reactors" within a population, all given the same dose and demonstrating the same degree of a specific biological effect, while effect designates a defined biological reaction, which in some cases may be measurable on a continuous scale.

These concepts are most easily visualized by considering effect as the change (which in some cases may be graded) due to a dose within an individual and response as the number of different individuals showing a specific effect. However, effect does not have to be limited to observations on individuals. Just as the effect value plotted graphically against dose may be the average value of replicates on an individual, it may also be the average value for the different individuals of the population given that dose. Further considerations are taken up by Pfitzer (1975).

When one wishes to enumerate all types of effects at each of several specific doses, a single curvilinear expression is not appropriate. In such cases there must be a dose-response curve for each effect. If this is not feasible, a tabular representation listing the effects and, when possible, their magnitude, at various dose levels may be useful.

Much of the experimental data pertaining to the toxicology of metals will be obtained as measurements of graded effects on individuals within a test population. Nevertheless, these data may often be converted to expressions of response for the population as a whole. For example, one may measure the increase in urinary ALA excretion in individuals receiving certain doses of lead but one may express the results as the increasing percent of the population showing above normal values with increasing lead dose or period of lead exposure. This practice for expressing response is justified especially by the recognition of the variation, often very wide, of the reaction of individuals within any population to a specific dose of a chemical. Such variations depend not only on well recognized characteristics as age, sex, race, health status, body weight, and diet but also an interaction of metals or other substances in the environment. It will often be impossible to separate populations into small groups based upon these characteristics, but their potential influence must not be forgotten.

When one deals with a human population, the deviation in response from the normal occurrence of a certain symptom or sign will strongly help to ascertain the possibility of detecting an effect by epidemiological methods. The specificity of the methods and the type of statistical analysis as well as a normal occurrence of the symptom in question, will determine the quantitative percentage that will be relevant in this context. It is also important to decide whether a deviation from the normal value which is statistically significant is also biologically so.

As mentioned earlier in this section it is generally believed that the dose-response curves displaying the frequency of a certain effect in relation to dose will have a sigmoid shape when the background frequency of the effect in question in the population has been subtracted. This classical dose-response curve is the cumulative form of a normal distribution. This curve is based on the "law of biological random variation" and it has been shown to be valid for e.g. the distribution of lethal doses of various compounds. In empirical studies, depending on which dose interval is under investigation, the resulting dose-response relationship may imitate a semi-logarithmic or linear relation.

Generally, dose-response relationships for metals have not been reported as dose-response curves. Data have usually not been collected in a way suitable for statistical analysis, or even when they were so collected such analysis has not been attempted. In Figure 1 dose-response curves are displayed for various effects of $MeHg^+$ from the epidemic in Iraq (Bakir et al., 1973; Clarkson and Marsh, 1975). A logarithmic dose scale was used in those reports. Whenever possible, such distributions should be given for each effect. The determination of the type of distribution is of importance, since, if the distribution is log-normal, it will be possible to make a more accurate estimate of the doses which give rise to low level response.

A metal or metal compound may cause effects of increasing severity as well as an increasing response for each effect when the level of exposure increases. Clarkson and Marsh (1975) have illustrated this well, giving a separate dose-response curve for each effect (Figure 1). Their data also provide an example of the difficulties in assessing a low response by clinical observations. Under the assumption that the dose-response curve is correct, it also depicts another essential point. It is evident from the figure that if one takes, for example, parasthesia as the effect and 15% as the response, a dose corresponding to a body burden of about 30 mg would give rise to this response. This of course does not mean that one could not find individuals with a higher dose who do not display paresthesia. In 85 persons out of 100 ran-

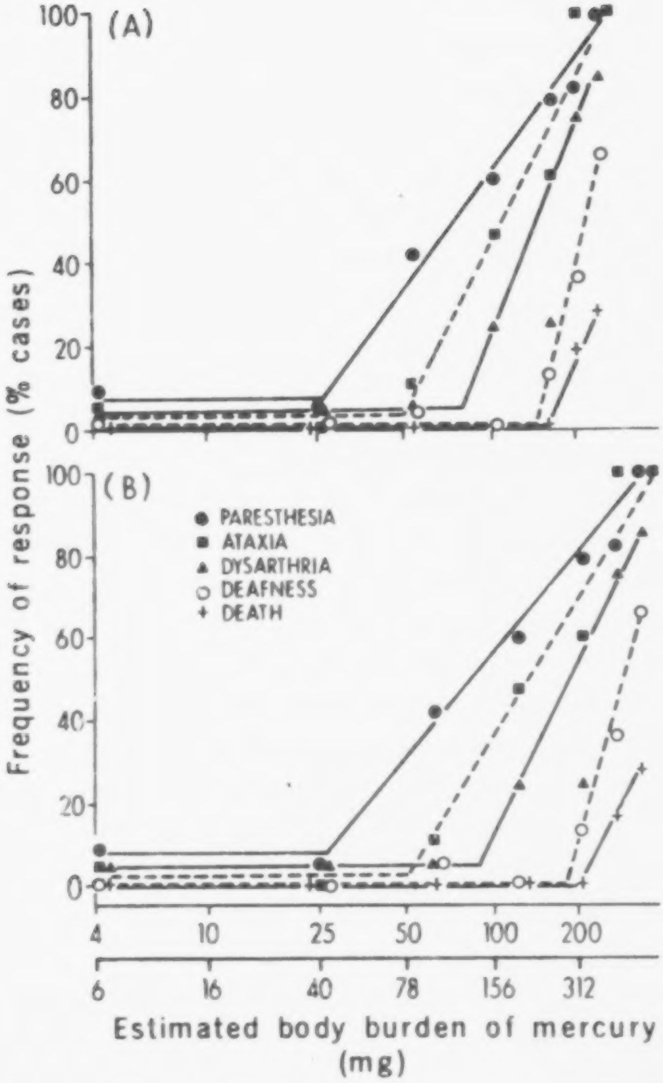

Figure 17. The relationship between the frequency of signs and symptoms and the estimated body burden of $MeHg^+$ (a) at the time of onset of symptoms and (b) at the time of the cessation of ingestion of $MeHg^+$ in bread. The two scales on the abscissa are for body burden of $MeHg^+$ calculated as described by Bakir et al. (1973) from linear regression lines (upper scale) and from the Miettinen line (lower-scale). (From Bakir et al., 1973.)

domly selected, this would be the case. In order to prove that 30 mg is too low an estimate of the given response rate of 15%, it would be necessary to show the existence of a statistically significant higher percentage than 85% that are nonaffected at the dose corresponding to a body burden of 30 mg. (Further considerations by Nordberg and Norseth, 1975; Kjellström, 1975a).

Sufficient epidemiological observations are often not available for an assessment of a dose-response curve. This may be true especially in long-term exposure, for which the quantitative assessment of exposure during different time periods is particularly difficult. In such cases, provided data on the metabolic model as well as on the threshold for critical effects are available, a mathematical approach may serve to derive a dose-response relationship. Such an approach has been used in connection with the evaluation of health hazards from long-term exposure to cadmium (Kjellström, 1975b) and from varying exposures to methylmercuric compounds (Berglund et al., 1971). Such calculations should take into consideration, if possible, the interindividual variability in the metabolic model and in the critical concentration in the critical organ (Nordberg and Strangert, 1975).

IV. 5.2 Cadmium: dose-effect and dose-response relationships

Critical organs have been discussed in IV.4 where the lung was concluded to be the critical organ for short-term inhalation exposure and the kidney for prolonged low-level exposure.

IV.5.2.1 Dose-effect and dose-response relationships in critical organs; critical concentrations in critical organs in cadmium exposures

Pulmonary effects may be critical in inhalation exposures to cadmium. Data on the relationship between lung concentration of cadmium and its effects are not adequate for a meaningful dose-effect or dose-response evaluation. The only reasonably well defined evaluation of this sort that can be made is based upon the dose estimated from air concentrations; this evaluation will be given in the paragraph about dose-response relationships based on other types of data.

Renal effects are critical in low-level long-term exposure to cadmium. There is evidence from animal data that at kidney cortex concentrations above 200 µg Cd/g wet weight, tubular dysfunction and/or histopathological kidney changes occur (Bonnell et al., 1960; Axelsson et al., 1968; Stowe et al., 1972; Suzuki, 1974; Nomiyama et al., 1975; Kawai et al., 1975). Some data support the occurrence of an effect already at lower concentrations (Piscator and Larsson, 1972; Kawai et al., 1975).

Kawai et al. (1975) found a correlation between renal cadmium concentration and severity of lesions under a constant exposure situation. However, when the exposure varied, the threshold concentration in the kidney for renal tubular damage also varied. Kawai and associates also pointed out that the time of exposure and the duration after exposure are important for the development of chronic lesions in the renal tubules.

Nordberg (1972a) and Suzuki (1974) reported data for different animals indicating that at the time at which cadmium-induced tubular changes appear, cadmium cccumulation in kidney cortex levels off. Kawai and Fukuda (1974) presented data showing the same tendency. At the breaking point, the rate of urinary excretion of cadmium changes and cadmium concentration in renal cortex decreases. On the other hand, if exposure continues, liver cadmium accumulation may continue even after kidney cortex cadmium concentration has decreased (Kawai and Fukuda, 1974; Suzuki, 1974; Friberg et al., 1974). Bonnell et al. (1960) and Nomiyama et al. (1973a, 1974) showed that at high dose levels liver cadmium concentration may even decrease. From the animal data, it may be concluded that cadmium concentration in kidney cortex, at the time at which more pronounced renal damage has already occurred, is not a good indicator of the individual critical concentration or total accumulated exposure. If, in humans suffering from renal cadmium injury, the total absorbed dose cannot be calculated from data on exposure, concentration of cadmium in liver is probably a better indicator of total absorbed amount of cadmium than is renal cortex concentration.

Information about morphological changes, proteinuria, and cadmium levels in kidneys and liver of human beings are on hand from about 30 autopsies and biopsies of persons exposed to cadmium. A detailed account of these data has been given by Friberg et al. (1974). It is evident that a detailed dose-effect or dose-response curve cannot be established with this limited amount of data. In persons with pronounced kidney damage the renal concentrations of cadmium were low, whereas in persons with no or slight renal changes, cadmium levels in kidney cortex with one exception varied between 180 and 450 μg/g wet weight.

Nomiyama (1973) reported data from 5 persons in an area with cadmium exposure. Three persons showed liver values higher than those commonly found in Japan (Tsuchiya et al., 1972a). Of those three, one (without proteinuria) had a kidney cortex concentration of 264 μg Cd/g wet weight, whereas another (with proteinuria) had 29 μg Cd/g wet weight. Proteinuria was not studied in the third person, who was highly exposed to cadmium for only 7 months and whose kidney cortex level was 134 μg Cd/g wet weight.

Friberg et al. (1974) concluded on the basis of available animal and human data that "it is considered justified to start out from a value of 200 μg/g wet weight in renal cortex, when discussing which exposures can bring about cadmium-induced, detectable renal dysfunction in man. This of course does not mean that 200 μg/g would give rise to renal tubular dysfunction in all persons exposed." It is thus understood that this concentration refers to a response of a part of the population displaying average or more than average sensitivity to cadmium. The magnitude of the inter-individual variation in sensitivity is not possible to establish more precisely at present.

This meeting agreed that 200 µg/g may be considered as a tentative critical concentration estimate in human kidney cortex, the validity of which will have to be assessed subsequent to further, greatly needed studies in humans. Animal data indicate that renal effects may be seen even at lower concentrations.

IV.5.2.2 Estimation, based on metabolic models, of necessary cadmium exposure in order to reach 200 µg/g in renal cortex

The shortcomings of the metabolic models for cadmium have been pointed out in section IV.3, and the uncertainties in defining a critical concentration for detectable renal tubular effects were alluded to in the foregoing paragraph. Even so, it can be worthwhile to illustrate how the models work..

Calculations of exposure situations giving rise to the assumed critical concentration of 200 µg/g wet weight in the critical organ, the kidney cortex, have been presented by Friberg et al. (1974), Nordberg (1974) and Kjellström (1975b). A summary of calculations made from entering data, assumptions and two different biological half-times into these models is presented in Table 8. The biological half-time has been estimated to be over 38 years (Kjellström et al., 1971; Kjellström, 1971) and about 18 years (Tsuchiya and Sugita, 1971). These calculations are admittedly tentative but are a good foundation for future research. They need to be verified by direct observations or re-evaluated if better metabolic models are found.

Table 8

Cadmium exposure necessary for reaching a kidney cortex concentration of 200 µg Cd/g using different alternatives for biological half-time in kidney cortex and exposure time (compiled from data by Friberg et al., 1974.)

		Estimated biological half-time in kidney cortex	
		[x]	[xx]
Basis of calculation[xxx]		38 years	18 years
	Exposure time (years)	Daily retention (µg)	
Constant daily retention during whole exposure time	10	36	39
	25	16	20
	50	10	13
		Industrial air concentration ($\mu g/m^3$)	
25% pulmonary absorption, 10 m^3 inhaled per work day, 225 work days/year	10	23	25
	25	11	13
		Daily cadmium intake (µg)	
Food exposure for 50 year old person (2500 cal/day) (4.5% retention) (changing caloric intake by age accounted for)	50	250	360
		Corresponding average concentration in foodstuffs (µg/g)	
Total amount (w.w.) of food/day 300 g	50	0.8	1.2
600 g		0,4	0,6
1000 g		0,25	0,35

[x] Kjellström, 1971

[xx] Tsuchiya and Sugita, 1971

[xxx] Assumptions: one third of whole body retention reaches kidney and kidney cortex concentration 50% higher than average kidney concentration.

Based on a suggestion from Professor Kitamura, some members of the present group expressed a different opinion. They considered that in order to calculate the time necessary for accumulation of the level 200 µg Cd/g in the renal cortex (Table 8), it was necessary that the biological half-time of that organ section be known. Specific figures such as 18 years or 38 years used in Table 8 were only apparent biological half-times calculated from kidney concentration of autopsy samples from the U.S.A. and Japan and could not therefore be considered applicable in the calculation of accumulated levels, if inter-organ redistribution of cadmium occurred in the body.

IV.5.2.3 Dose-effect and dose-response relationships for cadmium based on direct observations of exposure and effects

IV.5.2.3.1 Pulmonary effects

Acute effects in the form of pneumonitis as well as chronic effects in the form of emphysema have occurred in industrial exposures. When it comes to evaluating dose-response relationships, considerable difficulties exist, primarily depending upon the paucity of data concerning exposure. In an inhalation study on cadmium fume (0.2 µ AMD), LC_{50} values on rats were about 25 mg/m^3 for 30 minutes' exposure (Yoshikawa et al., 1974).

Friberg et al. (1974) reviewed the existing data and concluded that for human beings, fatal exposure to cadmium oxide fumes is not higher, but probably lower than about 2,500 mg/m^3 x min (corresponding to about 5 mg/m^3 for 8 hours' exposure). Interindividual variability has not been possible to evaluate.

For associations between long-term exposure and emphysema, the data are even more limited. Friberg et al. (1974) concluded that a prolonged industrial exposure to about 0.1 mg/m^3 of cadmium oxide fumes might well be considered hazardous with reference to emphysema. They based their conclusions primarily on reports by Bonnell (1955), Buxton (1955), King (1955) and Kazantzis (1956). Recent data from Lauwerys et al. (1974) suggest effects on the lungs of tobacco smoking workers at even lower occupational exposure to cadmium dust (66 μg Cd/m^3). The average tobacco consumption among these workers was 13 ± 1.5 cigarettes per day.

IV. 5.2.3.2 Renal effects

In Table 9 data from different epidemiological studies on cadmium-exposed workers are summarized. Most of the reported air concentrations are based on very short sampling periods although the exposure times are very long. By combining the data from Bonnell (1955) and Bonnell et al. (1959) it was possible to compare exposure time and response for about 150 factory workers. A higher prevalence of proteinuria was seen after about 10-20 years of an estimated exposure to cadmium fume of 50 μg/m^3 air.

Harada, A. (1973, 1974) studied 19 workers in a cadmium pigment factory,and reported proteinuria (trichloracetic acid method) among three of five workers with more than 10 years of exposure. The average exposure is difficult to estimate but Harada, A. (1973), after having made determinations in the air, calculated 114 μg Cd/m^3 for the worker with the longest exposure and 50 μg/m^3 for a group of newly employed workers (Harada, A., pers. comm.). When a more sensitive electrophoretic method for the analysis of tubular proteinuria was used, seven out of eight workers with an exposure time over 5 years had positive findings.

Lauwerys et al. (1974) did not find any difference in the prevalence of abnormal urinary electrophoretic patterns between controls and workers exposed to 134 μg CdO dust per m^3 at an average 8.6 years of exposure. Significantly

Table 9

Epidemiological data from industrial exposure situations useful for dose-response evaluations (data compiled from Kjellström, 1975b)

Reference	Effect studied	Estimated air concentration	Exposure time	Calculated response or effect
Bonnell (1955) and Bonnell et al. (1959)	Proteinuria (SA or TCA method)	40-50 µg/m^3 CdO fume	Controls	1/60
			< 9 years	6/22
			9-13 years	7/15
			14-18 years	9/19
			19-23 years	8/14
			>23 years	7/30
Harada (1973) (1974) and personal communication	Increased globulins in electrophoresis	Average among some workers= 50 µg/m^3. (Time weighted average)	<5 years	1/11
			>5 years	7/8
	Proteinuria (TCA method)	Average for Worker with longest exposure time= 114 µg/m^3 (range among air samples 24 - 1220 µg/m^3).	<10 years	0/14
			>10 years	3/5
Lauwerys et al. (1974)	Abnormal urinary electrophoretic pattern (as defined by the authors)		Controls	0/22
		Total CdO dust 134 µg/m^3 respirable fraction 88 ug/m^3	Average 8.6 years (range 0.6-19.6)	0/27
		Total CdO dust 66 µg/m^3 respirable fraction 21 µg/m^3	Average 27.8 years (range 21-40 years)	15/22

Table 9 (continued)

Epidemiological data from industrial exposure situations useful for dose-response evaluations (data compiled from Kjellström, 1975b)

Reference	Effect studied	Estimated air concentration	Exposure time	Calculated response or effect
Horstowa, Sikorski and Tyborski (1966)	Proteinuria (method not given)	Total dust 130 - 1170 μg/m^3	1-12 years	7/26
Friberg (1950)	Proteinuria (Heller method)	Total CdO dust 400-15000 μg/m^3	1- 5 years	0/26
			6-10 years	1/4
			11-15 years	3/9
			16-20 years	6/12
			21-25 years	3/9
			>26 years	6/9
Piscator, 1962a	Total quantative determination of urinary protein (biuret method, using Tsuchiya's reagent as precipitating agent)	40 workers from the study by Friberg, 1950 who were not exposed since that study	1-40 years	100 mg/24 hours at average 3 years exposure and 1000 mg/24 hours at 34 years
Adams et al., (1969)	Proteinuria (SA-method)	Total dust 500 μg/m^3	>5 years time for development of proteinuria= 7-24 years	20 %

higher prevalence was found among workers exposed to 66 μg CdO dust/m^3 for 27.8 years (average).

Other studies (Table 9) have shown renal effects at higher exposure levels. A dose-response curve based on a combination of all data cannot be constructed because of the different methods used for analysis of both exposure and effect.

An increased response as well as effect (increased concentration of protein in urine) is seen with increasing exposure time in some studies, and proteinuria may persist after exposure has ceased (Piscator, 1962b, 1966). When sensitive methods are employed, tubular proteinuria can be detected already after a few years at estimated exposure levels of 50 μg/m^3 (Harada, A., 1973, 1974). These data support Tsuchiya (1967) who found that industrial air concentrations of cadmium dust and fumes must be kept below 50 μg/m^3 in order to prevent cadmium-induced tubular proteinuria after prolonged exposure.

Reports used for dose-effect relationships among occupationally exposed workers have been collected to some extent in terms of the concentrations in the working environment. However, one must be careful when these results from occupationally exposed workers are applied to the general population since the sensitivity to cadmium may be different in different population groups.

Friberg et al. (1974) reviewed the epidemiological studies made in areas of Japan where the general population has been exposed to cadmium via food. The same data and some additional data were discussed by Kjellström (1975b) from the dose-response point of view. Friberg et al. (1974) concluded "considering all the data together, there can be no doubt that cadmium plays a role in the etiology of elevated prevalence of proteinuria in heavily exposed areas." The present daily intake in areas where health effects have been seen was estimated by Friberg et al. (1974) to be 200 to 300 μg or higher. The studies in Japan were not performed for dose-response evaluation of renal effects but for detection of Itai-itai patients. Therefore, the dose evaluations, based mainly on cadmium concentrations in rice, are only approximate. Analysis of cadmium in feces may be a promising method for cadmium intake estimates in epidemiological studies (Tati et al., 1975).

When Watanabe et al. (1974), in their studies in Japan, give a value of, for instance, 0.3 or 0.4 μg Cd/g in rice, this does not of necessity reflect the actual exposure levels over a period of many years. Hence, such values cannot be readily applied to dose-response relationships. The intention was to use cadmium level in rice only as an "indicator" for evaluating cadmium intake; it is recognized that the rice levels document only the present level of exposure.

Kjellström (1975b) estimated on the basis of epidemiological data and his metabolic model that somewhere between 0.4 and 0.6 μg Cd/g average concentration in basic food would be high enough to induce tubular proteinuria in a significant part of the population. He assumed that the intake of rice was 300 g/day and that half the cadmium intake came from rice. Tati et al. (1975) presented data showing that the average daily cadmium intake in a non-exposed Japanese population was about 60 μg. Kjellström's estimation implies that the necessary average intake for causing tubular proteinuria in a population is 240-360 μg.

Friberg et al. (1974) concluded that the data from autopsies, animal experiments, theoretical accumulation models, and epidemiological evidence from the industrial and general environment agree well as to the relationship between cadmium and renal effects.

IV. 5.2.4 Factors influencing dose-effect and dose-response relationships for cadmium

Metals suspected to have an influence on dose-response relationships for cadmium in human beings include zinc, selenium, and possibly copper. As mentioned earlier, protein and calcium content of the diet will influence gastrointestinal absorption of cadmium. An interaction between cadmium and lead has been suspected, but not confirmed. Some experimental data on influence of selenium, copper and some other metals will be presented in section IV.6. The following discussion will be devoted mainly to the influence of zinc on cadmium toxicity.

It has been postulated that the renal dysfunction occurring at higher renal levels of cadmium may be caused by interference of cadmium with zinc-metalloenzymes (Friberg et al., 1974), and the renal content of zinc can thus be an important factor when discussing dose-response relationships. Leucine aminopeptidase is a zinc metalloenzyme, which has been isolated from renal cortex of pigs (Himmelhoch, 1969; Lisowski et al., 1970). Cousins et al. (1973) found that in cadmium-exposed pigs the activity of this enzyme was decreased in the renal cortex.

In human beings it has been shown that the "normal" accumulation of cadmium in the renal cortex is accompanied by an equimolar increase in zinc (Piscator and Lind, 1972), whereas no change in copper concentrations takes place. There are few data on both cadmium and zinc in renal cortex from human beings with excessive exposure. Piscator and Lind (1972) found that a 24-year-old man with occupational exposure to cadmium had a cadmium concentration in renal cortex of 195 μg/g wet weight and a zinc concentration of 67 μg/g wet weight. The averages in a "normal" group, mean age 44 years, were 30 and 52 μg/g respectively.

In normal horses without excessive exposure, an equimolar increase in zinc was observed in the renal cortex at cadmium concentrations in the same range as those found in human beings, e.g. <75 μg/g wet weight. At higher cadmium levels up to about 250 μg/g wet weight, zinc did not increase to the same extent. In fact, at concentrations of cadmium around 200 μg/g, e.g. about 3 times higher than the upper "normal" limit in human beings, zinc concentrations had increased by only 50% (Piscator, 1974). This indicates that cadmium affects zinc kinetics in the kidney at concentrations below the critical concentration in renal cortex.

The equimolar relationship between cadmium and zinc in equine renal metallothionein under normal conditions (Kägi et al., 1974) may explain the "normal" equimolar relationship in the renal cortex. In experimental animals, the ratio between cadmium and zinc in metallothionein will increase under exposure to cadmium. This may be related to the changed relationship between zinc and cadmium found at higher renal cadmium concentrations.

IV.5.2.5 Summary of dose-effect and dose-response relationships for cadmium and its compounds

Only solitary data exist on renal concentrations of cadmium in human exposures and even dose-response relationship data in animals are scarce. Various metabolic models have been proposed to estimate the critical levels of cadmium in the renal cortex. On the basis of available studies, a level of 200 μg Cd/g wet weight has been suggested as a tentative critical concentration in the renal cortex for human beings. At this concentration there is a response of a part of the population that displays average or more than average sensitivity to cadmium. The final validity of this value needs to be verified by further epidemiological studies on occupationally exposed workers and residents of cadmium-polluted areas, particularly regarding the relationship of cadmium to coexisting kidney levels of zinc and metallothionein.

The limited data base, built upon observed human exposures up to this date, indicates that in long-term inhalation exposures, air concentrations lower than 50 μg Cd/m^3 are necessary to prevent cadmium-induced proteinuria. In peroral exposures, the corresponding figure for daily intake has been suggested at the level of 200-300 μg Cd/day in adult persons living under satisfactory nutritional conditions.

IV.5.3 Dose-effect and dose-response relationships for lead (inorganic lead compounds)

IV.5.3.1 Introductory remarks

It has been concluded earlier (section IV.3.3) that a useful metabolic model for lead describing the relationships between exposure, body burden, and concentrations in critical organs and index media, is not available, nor are there many data on concentration-effect relationships in the organs considered critical in lead exposure (bone marrow, nervous system, kidney - see discussion in Section IV.4). For these reasons the discussion in this section will be mainly devoted to the relationships between the blood lead concentration (Pb-B) and hematological, nervous, renal and other effects, under the assumption that the blood lead concentration reflects soft tissue concentrations.

Valid conclusions require valid measurements. Even disregarding that Pb-B is only an indirect measure of dose, or of exposure, it is well known that lead measurements are extremely vulnerable to methodological error. This vulnerability has created great confusion regarding both dose-effect and dose-response relationships. In general, older data tend to be higher than those obtained in the 1960's and 1970's, but considerable variations in accuracy and precision remain, even between experienced laboratories, as shown by some recent comparative studies (Donovan et al., 1971; Berlin, A., et al., 1973, 1974). Under these circumstances, it is small wonder that discrepancies are found when all literature to date is examined. In the following review only data from laboratories or research institutes with a good methodological level are considered; yet, variations of 10 to 20 μg Pb/100 ml blood will become apparent.

Quite naturally, the validity (precision and accuracy) of the measurements of effects is as important as that of the dose. Interlaboratory comparisons have shown different degrees of validity for different responses, e.g. the validity of ALA-D measurement is relatively high, and that of ALA moderate to high as compared to lead in blood measurements (Berlin, A., et al., 1973). Quantitative comparative data for other parameters of response are scanty.

IV.5.3.2 Dose-effect and dose-response relationships in critical organs and indicator (or index) media

IV.5.3.2.1 Hematological effects

The hematological effects of lead are the only ones extensively studied by biochemical methods, and for which the mechanism of a lead action is known in reasonable detail (see Chapter 4). These effects are also the only ones for which relationships to dose have been established with any satisfactory accuracy and, even so, the only relationships defined are those to lead in blood. Direct relations between lead concentrations in the bone marrow and effects are unknown. However, Wada and Ohi (1972) have observed depressed ALA-D and heme-S activities and reduced heme synthesis in erythroid cells from the bone marrow of lead workers whose blood lead concentrations were in range of 40-90 μg/100 ml.

The earliest effect related to hematopoietic tissue is inhibition of ALA-D which becomes measurable at about 10 to 20 μg Pb/100 ml blood (Hernberg et al., 1970). Erythrocyte ALA-D is also inhibited in vitro by a number of substances, e.g., EDTA,mercury, copper, silver, manganese and cobalt (Hernberg and Nikkanen, 1972). There are data on in vivo inhibition by ethyl alcohol (Millar, 1973), but the inhibitory action of all these agents is less than that of lead. There exists a highly significant semilogarithmic inverse relationship between lead in blood and ALA-D activity. When high lead levels are included, the correlation coefficients are as high as 0.8-0.9. In a large study by Tola (1973) the level for detection of a slight inhibitory effect upon ALA-D was 10-20 μg Pb/100 ml blood. Similar results have been reported by Haeger-Aronsen et al. (1971) and by Sakurai et al. (1974). However, another study (Hernberg et al., 1970) has not demonstrated such a threshold. It is not yet clear if a linear relationship persists below the 10-20 μg Pb/100 ml level in blood, or if a plateau is formed. Sassa et al. (1973) have proposed, on the basis of kinetic studies, that inhibition of ALA-D activity would not occur below 15 μg Pb/100 ml of blood.

The activity of ALA-D is almost totally inhibited at blood lead levels of 70-90 μg/100 ml. For adults in industry, the relationship between lead in blood and ALA-D is roughly the same regardless of whether the exposure is long-term steady state, acute, or has been terminated (Tola et al., 1973). A partial ALA-D inhibition cannot be regarded as a deleterious effect in itself.

A minor depression (approx. 30%) of the ALA-D activity occurs in 50 percent of the population at approximately 30 μg/100 ml lead in blood. At a blood lead level of 50 μg/100 ml, 50 percent of the population shows about 65 percent reduction in the ALA-D activity. A 90 percent reduction occurs in 50 percent of the population at blood lead levels around 75 to 80 μg/100 ml (Tola, 1973).

A threshold of about 35 to 40 μg Pb/100 ml of blood for an increase in the excretion of ALA-U has been reported by some authors (Selander and Cramér, 1970; Haeger-Aronsen et al., 1971; Tola et al., 1973), and one of about 50 by others (Sakurai et al., 1974; Chisolm et al., 1975). Such slight differences probably stem from methodological discrepancies. Above this threshold the concentration tends to increase rapidly. However, the correlation of ALA-U with Pb-B is not as close as that for the ALA-D activity. Usually, the correlation coefficients have been between 0.5 to 0.7 for blood lead values in the range of 40 µg/100 ml and above (Selander and Cramér, 1970; Tola, 1973). Approximately 50 percent of a population with a blood lead level of 50 µg/100 ml exhibited ALA-U values greater than 5 mg/l urine, and at 65 µg Pb/100 ml blood 50% of ALA-U values exceeded 10 mg/l urine (Zielhuis, 1975a).

Urinary coproporphyrin (CP-U) excretion also starts increasing at approximately 35 to 40 µg lead/100 ml blood, and it is thus almost as sensitive as ALA-U to the action of lead (Tola et al., 1973). However, increase of CP-U may also be due to other factors than lead, e.g. alcohol abuse, liver disease, porphyrias, etc. This parameter is thus less specific than ALA-U.

The concentration of PP IX in the erythrocytes (FEP) starts to increase at Pb-B levels of about 25 to 35 µg/100 ml in children and women, and at about 30 to 40 µg/100 ml in men (Stuik, 1974; Zielhuis, 1975a). The difference between women and men may be due to the fact that PP IX is sensitive to iron deficiency; in general, women have smaller stores of iron. Elevated PP IX levels persist long after the cessation of exposure; thus the correlation to Pb-B cannot always be expected to be as close as those for the parameters discussed above. However, in steady state, coefficients of up to +0.91 have been demonstrated (Sassa et al., 1973). Chisolm et al. (1974) also found a relationship between blood-lead concentration and protoporphyrin concentration in children. The increase occurred at somewhat lower blood lead concentrations in children with mild anemia and was interpreted as an effect of low iron intake.

No studies of suitable epidemiological design have as yet come forth which would allow a close approximation of the dose-response relationship for FEP in men, women and children. For a further discussion on this topic, see Zielhuis (1975a).

Reticulocytosis becomes measurable at Pb-B's between 60 and 80 µg/100 ml. In this range a shortening of erythrocyte life span begins as well (Hernberg et al., 1967a). The degree of anemia correlates poorly, if at all, with Pb-B. However, a slight drop in the hemoglobin level has been shown at Pb-B levels of about 50 to 80 µg/100 ml in new lead workers during their first three to four months of employment (Tola et al., 1973). Obvious anemia usually does not develop when Pb-B is below 80 µg/100 ml. At higher Pb-B levels all abnormalities become more and more pronounced, but inter-individual variation is large.

IV.5.3.2.2 Neurological effects

IV.5.3.2.2.1 Central nervous system effects (Encephalopathy)

As discussed in section IV. 5.3.1.2.1, encephalopathy resulting from lead exposure usually requires more intensive and more prolonged exposure than that giving rise to hematological effects. The exact mechanism behind lead-elicited acute encephalopathy is not known. It is difficult to ascertain whether the effect is usually a result of repeated acute incidents of lead exposure or a result of continuous exposure, causing a high level of lead in the CNS. The onset and course of acute lead encephalopathy are always unpredictable. Although high blood lead concentrations -usually much above 100 µg/100 ml - are required, other severe symptoms such as gastrointestinal colic are not always present. It is hence obvious that neither clear-cut dose-effect nor dose-response relationships for encephalopathy may be stated at the present time.

It may be that children suffer other CNS effects at levels even lower than 100 µg/100 ml. It has especially been discussed whether long-term low level exposure to lead in children can give rise to an increase in frequency of mental retardation (Perlstein and Attala, 1966) or hyperactivity (David et al., 1972; David, 1974). Albert et al. (1974) found an increased frequency of deleterious effects in a follow-up of children without diagnosed lead poisoning, but with a history of blood lead levels above 60 µg/100 ml. Data on concentrations of lead in the CNS in human beings with various types of lead-induced CNS-effects are not available. Such data are lacking for experimental animals as well.

Lead readily crosses the placenta, and the correlation between cord and maternal blood lead levels is good. Ratio of maternal and fetal blood lead is near unity regardless of differences in hematocrit. Therefore although no data are available, it must be said that an increased body burden of lead in women of child-bearing age may mean a possibility of effects on the central nervous system in the fetus. This may hold true even in the absence of evidence of lead intoxication. This question needs systematic study.

IV.5.3.2.2.2 Peripheral nervous system manifestations

While dose-response relationships for the clinical manifestations of lead effects on the peripheral nervous system are incompletely established, there are better data available concerning subtle effects, which have been assessed by electrophysiological techniques. It has been possible to demonstrate effects on the peripheral nervous system in lead workers whose blood lead levels were in the order of 80 to 120 µg/100 ml and who had varying degrees of lead poisoning, but who were completely without any clinical neurological symptoms or signs (Sessa et al., 1965; Catton et al., 1970; Seppäläinen and Hernberg, 1972). The abnormalities consisted of a slowing of the conduction velocity of the nerves of the upper limbs, particularly in the slower fibers and in the distal portion, and electromyographic abnormalities such as fibrillations and diminished numbers of motor units upon maximal contraction. Similar abnormalities, although of a slighter degree, have been demonstrated in a group of lead workers who were exposed between 1 and 23 years, whose blood lead values had never exceeded 70 µg/100 ml, and in whom no signs of lead poisoning had been noticed. At examination the mean blood lead level was 40 ± 9 µg/100 ml with a maximum of 65 µg/100 ml (Seppäläinen 1974; Seppäläinen et al., 1975).

In Figure 18 it is proposed that slight peripheral neuropathy may occur in a small percentage of the population already at blood lead levels of about 50 µg/100 ml. About 50% of the population would be expected to show this effect at lead levels of about 120 µg/100 ml and 90% would probably experience at least slight damage at lead levels of about 150 µg/100 ml. At this uppermost level, a few percent of the population could be expected to show severe damage. However, more exact dose-response data are required to confirm these assumptions (Zielhuis, 1975b). Data relating lead concentrations in nerves or nerve cells to effects are not available for human beings or animals.

IV.5.3.2.3 Renal effects

Data on the renal effect of lead are incomplete, and the dose-response relationships are even less well-established than those for the neurological effects. Neither human nor animal data have been interpreted in such a way as to show a critical organ concentration for renal effects of lead. When more extensive information is available, the older data can perhaps be reinterpreted with that purpose in mind. Goyer et al. (1970) found intranuclear inclusion bodies in the kidneys at a renal lead concentration of 10 µg/g in rats. About 60 µg Pb/g kidney will be required for aminoaciduria and renal edema to appear in rats given lead in drinking water.

Renal effects of lead have also been related to blood lead levels, but this relationship is incompletely documented. Goyer and Rhyne (1973) suggested that blood lead levels in the range 40-80 µg/100 ml will be associated with inclusion bodies in the renal tubular epithelium. At blood lead levels above 150 µg/100 ml Fanconi's syndrome may develop among exposed

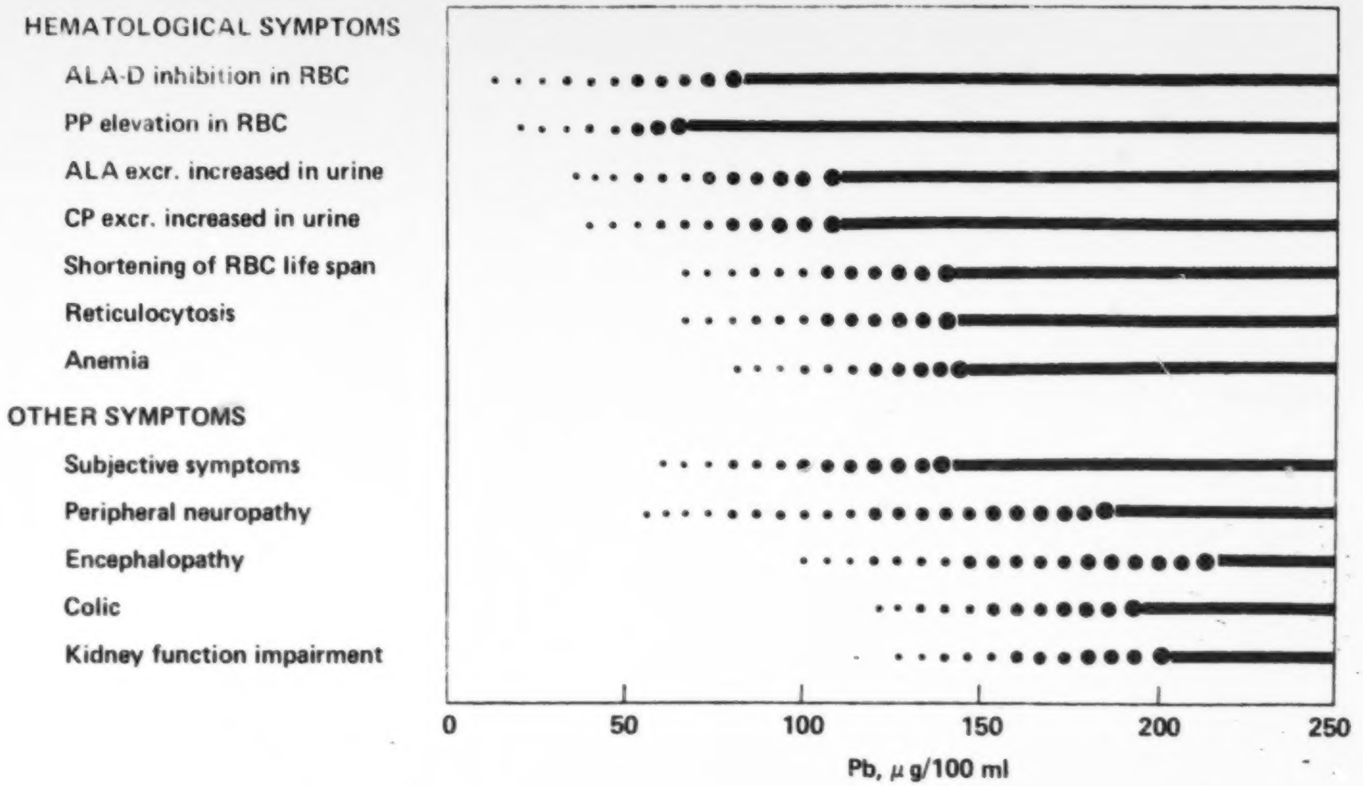

Figure 18. Relation between blood lead levels and the onset of a number of effects. (From Hernberg, 1975.)

children (Chisolm and Leahy, 1962), but the exact dose-response relationship is not known. In occupationally exposed workers, kidney function impairment, as shown by increased blood urea levels, may occur at blood lead levels of about 120 µg/100 ml in a small portion of the population (a few percent); about 50% of the population would suffer such impairment at a blood lead level of about 160 µg/100 ml, while more than 90% at 180 µg Pb/100 ml (Hernberg, 1975). However, these dose-response relationships for kidney function impairment are uncertain.

IV.5.3.3 Summary of dose-effect and dose-response relationships for inorganic lead compounds

Dose-effect relationships in critical organs of humans cannot be given because no substantial body of data exists on the concentrations of lead in the organs which it affects (i.e. bone marrow, nervous system, kidney) in conjunction with measurements of effects in these organs. There are substantial data relating the various effects to several ranges in blood lead concentration. The critical effects on heme synthesis (increased ALA-U and CP-U) begin to appear in the range of 30-50 µg Pb/100 ml whole blood but the range may be slightly lower for increased PP IX (FEP) to appear. Depressed ALA-D activity, depressed heme-S activity and reduced heme synthesis in erythroid cells from the bone marrow have been observed in lead workers with blood lead concentration in the 40-90 µg/100 ml range. Renal effects and acute clinical effects have generally been observed only at higher ranges of blood lead concentration. Limited data in humans strongly suggest that the CaEDTA mobilization test, which provides a measure of the biologically active lead, may be a better indicator in intact man of the concentration of lead in affected organs.

It should also be pointed out that the CNS may be the critical organ under certain circumstances, particularly in the very young child or the fetus. A discussion of this possibility has been undertaken (IV.4.3.1.2.1 and IV.4.3.3) but no precise dose-response relationships can be set up.

Recent studies suggest that a nerve conduction deficit detectable by electromyography might be the critical effect in certain adults with industrial exposure to lead. A preliminary estimation of dose-response relationships for this effect has been presented (IV.5.3.2.2.2).

IV.5.4 Mercury: dose-effect and dose-response relationships

IV.5.4.1 Mercury vapor

IV.5.4.1.1 Dose-effect relationships in critical organs and indicator (or index) media; critical concentrations in critical organs

On short-term exposure to high levels of mercury vapor, the lung is the critical organ. Following repeated or prolonged exposure to moderate levels of mercury vapor the brain will be the critical organ. Reliable data on concentrations of mercury in the lungs on short exposure to mercury vapor have not been found in the literature, nor is there any suitable diagnostic indicator (or index) media reflecting the concentration in the lungs after exposure to mercury vapor. Therefore, it is possible to provide neither dose-response nor dose-effect relationships for mercury concentrations in the lung. There are too few data about effects on the kidney after exposure to mercury vapor to establish a dose-effect relationship.

In animal studies, mercury concentrations found in the brain after exposure to mercury vapor have been reported by Ashe et al. (1953). They exposed rabbits to 6 mg Hg/m^3, 0.9 mg Hg/m^3 and 0.1 mg Hg/m^3 during 7 hours per day, five days per week during various periods. At mercury concentrations between 3 and 17 µg/g they found moderate histopathological changes and at levels between 1 and 2 µg/g definite but mild histopathological changes. At levels up to 0.5 µg/g no changes were observed. In studies by Berlin (1975), levels up to 8 µg/g in the occipital part of the cortex were observed without any definite pathological changes in the squirrel monkey. In Berlin's opinion, behavioral disturbances may have occurred in those monkeys.

Studies from the USSR have reported behavioral changes at exposures to mercury vapor of less than 0.1 mg/m^3, but conclusive data on brain concentrations in relation to these findings are not available. Friberg and Nordberg (1972) have summarized the Russian literature in this respect, especially studies by Trachtenberg (1969) in cats and by Kournossov (1962) on rats. The Russian data indicate that behavioral effects may occur at considerably lower concentrations in the brain than those giving rise to clear-cut morphological damage. This may be relevant when discussing dose-response relationships for mercury vapor.

IV.5.4.1.2 Mercury vapor: dose-response relationships

Data on dose-response relationships on exposure to mercury vapor are limited (review: Friberg and Nordberg, 1972; NIOSH, 1973). Smith et al. (1970) studied a group of 567 workers in the chlorine industry exposed to mercury vapor. They showed increasing prevalence of loss of appetite, loss of body weight, tremor, insomnia, shyness, and history of nervousness at exposure levels from 0.1 to 0.27 mg/m^3. Exposure in chlorine industries, however, includes chlorine as well as mercury.

IV.5.4.1.3 Use of metabolic models for establishing dose-effect relationships for mercury vapor

It is evident from the foregoing account that data, both with regard to the metabolic model and with regard to the critical organ concentration for mercury in the brain and lungs after vapor exposure, are too uncertain to allow any useful calculations on dose-effect and dose-response relationships. Further research is needed on this question and also on the relationship between concentration of mercury in blood and urine versus that in brain.

IV.5.4.2 Inorganic mercury compounds : dose-effect relationships in critical organs and indicator (or index) media; critical concentrations in critical organs

On exposure to mercuric salts regardless of whether inhaled as a dust or ingested, the kidney is the critical organ (section IV.4.3). Available evidence concerning relationships between concentration in the kidney and effects has been summarized by Berlin (1975). In case of known poisoning due to short-term exposure to mercuric salts, values between 10 and 70 μg Hg/g have been reported in human beings (see also IV.4.4.3). It seems from evidence to date that levels of mercury in the kidney between 10-40 μg/m give rise to slight to moderate changes whereas concentrations of over 100 μg/g are associated with severely damaged kidneys. However, the level at which effects occur is determined not only by variation in individual sensitivity but also by the rate of dosage, or, rate of accumulation in kidney tissue.

As mentioned in earlier sections there is no medium that is good for paralleling mercury concentration in the kidney; thus relationships between mercury concentration in indicator (or index) media and effects are not meaningful. Even so, it cannot be excluded that urinary excretion of mercury or blood mercury levels during conditions of no exposure may reflect mercury concentrations in the kidney. This has to be studied.

IV.5.4.3 Summary of dose-effect and dose-response relationships for mercury vapor and inorganic mercury compounds

Data of possible relevance to the critical organ concentration of inorganic mercury in the kidney and the critical organ concentration of mercury vapor in the brain have been reviewed, but it has been concluded that it is difficult to see any certain, exact relationship between the concentration and the response. However, the data at hand allow a very rough estimation of the approximate concentration connected with effects. For mercury vapor, also, effects on the brain displayed as behavioral changes have to be taken into account. Their dose-response relationships are difficult to evaluate on the basis of the critical organ concentration, because of the scarcity of data and because of the insecure experimental and analytical foundation on which they are built. Data on direct exposure-effect relationships do suggest that such changes may occur at considerably lower chronic exposures than those giving rise to histopathologically evident changes.

IV.5.4.4 Organomercury compounds

IV.5.4.4.1 Methylmercuric compounds

IV.5.4.4.1.1 Dose-effect and dose-response relationships in critical organs; critical concentrations in critical organs (critical body concentration)

As mentioned earlier, the critical organ system in poisoning by alkylmercuric compounds is the CNS in man and other primates. In lower animals like rabbits or rats, the peripheral nervous system or the kidney may be critical organs. Especially the rat, which suffers renal damage from exposure to methylmercuric compounds, is not a good model for studies on dose-effect relationships for $MeHg^+$. Caution should be exercised when using data from this species for evaluation of the dose-response relationships for other animals and man. The fetal brain is the critical organ during pregnancy. It has been stated above that $MeHg^+$ may affect the chromosomes at a low level, below that giving rise to detectable changes in the CNS. It was also said that sufficiently documented data on this possibility are not yet available. It is not possible, at present, to talk about a critical concentration for this type of effect.

An attempt will be made here to estimate the adult brain concentration associated with death and also the brain concentrations associated with signs and symptoms of methylmercury poisoning. Special attention will be paid to the more sensitive part of the population and thus to the lowest brain concentrations that may ever have been associated with symptoms. In these discussions about brain concentrations, data derived from studies on blood values, liver values, total intakes and body burdens will be used. The concentrations of $MeHg^+$ in brain associated with death in the more sensitive individuals of the population may be estimated from data derived from various sources. Studies on limited numbers of non-human primates (Nordberg et al., 1971a; Berlin, M. et al., 1975a, 1975b) indicate that the minimum lethal concentration is above 9 µg Hg/g wet weight and that the minimum concentration for signs is lower than 9 μg/g. Data on brain levels in human beings poisoned in the Minamata outbreak, as interpreted by a Swedish expert committee (Berglund et al., 1971), indicate that the average brain level at the time of onset of symptoms was 25 μg/g. In one patient, it may have been as low as 6 μg/g. The levels at the time of onset of symptoms probably correspond to maximum brain levels, since this patient most likely entered the hospital at about this time and therefore stopped consuming contaminated fish.

Magos et al., (1974c) reported on fifty autopsy specimens of liver from the recent Iraqi outbreak. The average level was 16.3 μg Hg/g wet weight, the lowest being 1.4 μg Hg/g wet weight. This level is so low that it is difficult to believe that methylmercury oompounds were the sole cause of death. The highest recorded value was 75.5 μg/g wet weight. Taking the average liver levels and assuming a ratio of 2 for liver to brain concentrations (Berglund et al., 1971), these data indicate an average brain level at death of 8 μg/g wet weight. Most of these patients died early in the outbreak, approximately 45 days after the exposure to mercury. Thus the maximum average brain level in fatal cases probably was about 16 μg/g wet weight. Examining the distribution of liver levels in those cases, and taking into account other possible causes of death, the lowest liver level in a fatal case of methylmercury poisoning was 10 μg/g. Correcting for a 45-day period after exposure, the maximum brain level in this case would be about 10 μg/g.

Bakir et al. (1973) reported estimates of maximum body burden of $MeHg^+$ in fatal cases in Iraq (Figure 17b). They noted that the probability of a fatal outcome rose slightly above a body burden of 200 mg in a 50 kg person, corresponding to 280 mg in a 70 kg person. Using the parameters of the metabolic model for $MeHg^+$ in man (section IV.3.3.3.4.3.1), this should correspond to a brain level of approximately 17 µg/g. An alternative estimation of the body burden also presented by Bakir et al. indicates a "threshold" lethal body burden of 312 mg corresponding to a brain level of 18 µg/g. Data obtained from the outbreaks in Japan and Iraq are in reasonable agreement on minimum lethal level in the brain (Table 10) indicating that this level is above 6 µg/g and probably lies in a range from 6 to 12 µg/g.

Data from animal tests and from the outbreaks of poisoning in Japan and Iraq allow estimates to be made of minimum brain concentrations of $MeHg^+$ associated with signs and symptoms of poisoning. The studies on monkeys referred to above indicate the minimum brain level for signs and symptoms to be below 9 µg/g.

Analysis of data from the outbreak in Niigata (Berglund et al., 1971) indicates an estimated lowest blood level at the time of onset of symptoms of 200 ng/g. This would correspond to a brain level of about 1 µg/g. Most hair samples collected during the Niigata epidemic had concentrations above 200 µg/g corresponding to a blood level of about 670 ng/g. The hair data cast some doubt as to the minimum blood level of 200 ng/g. In the case of mild poisoning, an exact recollection by the patient concerning the time of onset of symptoms is not to be expected. The value of 200 ng/g estimated by back extrapolation may have been higher at the time of onset of symptoms.

The information reported in Figure 17 of this report (quoted from Bakir et al., 1973) indicates that the first effects of $MeHg^+$ become detectable at a body burden in the range of 25 to 40 mg in a 50 kg individual. This would correspond to brain levels of 2.1 to 3.4 µg/g in a 70 kg standard man. New data reported by Kazantzis (1975) support the dose-response data of Bakir et al. (1973). For example, Kazantzis

Table 10

Estimated Minimum Concentrations in Brain Associated with Signs and Symptoms and Death in Poisoning by Methylmercuric Compounds

Data Source	Concentration (μg/g)		n	Reference
	Signs & symptoms	Death		
Squirrel Monkey	<9	> 9	16	Berlin et al. (1975b)
Minamata (Brain autopsy)		> 6	12	Berglund et al. (1971)
Niigata (blood levels)	1		17	"
Iraq (liver autopsy)[†]		> 10	51	Magos et al. (1974c)
Iraq (body burden)[†]	2-3	> 17	125	Bakir et al. (1973)
Iraq (ingested dose)[†]	2-3		470	Kazantzis (1975)
Iraq (hair levels)[†]	4		93	Al-Shahristani et al. (1974)

n = number of individual observations.

[†] Brain concentrations calculated on the basis of the metabolic model for MeHg$^+$ in the standard man (see text).

reported on a population of 473 people for whom the average dose was 150 mg Hg ingested over a period of 32 days. Assuming a clearance half-time of 70 days for the whole body and using equation (3), it may be calculated that the average maximum body burden in the population was 125 mg. The frequency of symptoms in the population was 50%. In a study by Al-Mufti et al. (1974) the frequency of paresthesia was 26% in a total of 165 persons consuming 50-99 contaminated loaves (1.4 mg per loaf). The Bakir et al. (1973) dose-response curve indicates that the frequency of paresthesia at this body burden is between 50 and 60%. There is thus a close agreement between these two independent estimates. The Kazantzis study (Kazantzis, 1975) also points at a minimum brain level associated with symptoms of from 2 to 3 µg/g using the metabolic model.

Al-Shahristani et al. (1974) have compared signs and symptoms of poisoning in the Iraqi outbreak with maximum hair concentrations of mercury. These results indicated that the mildest cases of poisoning occurred at hair levels of 120 µg per g and above. Al-Shahristani et al. were able to compare hair concentrations to body burdens of $MeHg^+$ estimated from the ingested dose. These observations indicated that a hair level of 60 µg/g in the Iraqi subjects was equivalent to an average body burden of 0.8 mg/kg. According to the metabolic model this would be equivalent to a brain level of 3.7 µg/g.

In summary (Table 10), observations on the outbreaks in Japan and Iraq are essentially in good agreement, indicating that the minimum brain levels in adults associated with signs and symptoms fall in the range of 1-4 µg Hg/g wet weight.

It was mentioned above that the fetal brain is the critical organ in pregnant females, but there are at present no data in the literature on concentration of $MeHg^+$ in the fetal brain of exposed human or primate fetuses. A quantitative evaluation of this effect thus cannot be made.

IV.5.4.4.1.2 Methylmercuric compounds: dose-effect relationships based on observations of intake versus effects

Data have been presented in the previous section on critical and lethal brain concentrations of $MeHg^+$ in squirrel monkeys and adult humans. Comparable data are not yet available on the fetus.

The Iraqi studies on a human population exposed to methylmercuric compounds by ingestion of contaminated bread demonstrated a correlation between intake and severity of clinical manifestations of poisoning. Those who had consumed 65 mg $MeHg^+$ (range 35-100 mg because of the varying content of $MeHg^+$ in flour) had symptoms but no signs. 80% of those with signs had eaten more than approximately 130 mg, (range 70-200 mg) (Kazantzis, 1975). Correlation between intake of methylmercuric compounds and clinical evidence of toxicity was also reported (Bakir et al. 1973; Clarkson and Marsh, 1975). Although it is not possible to quantitate the degree of effect with the available data one may establish a rank order of these effects based on the dose required to produce 50% response of each effect.

In the Iraqi studies relationships between intake levels and blood and hair concentrations were also recorded. The relationship between the concentration of total mercury in blood and the amount of mercury ingested was established for a group of children aged 10 to 15 years and for a group of adults by Bakir et al. (1973). Correlation between intake of total mercury and hair concentration of total mercury was demonstrated by Kazantzis (1975).

In the latter study, the mean maximal hair concentration of total mercury for those who had eaten less than 50 loaves (approximately 1.3 mg/loaf, but could have varied between 0.5 mg and 2.0 mg per loaf or even more widely), 50 to 100 loaves, 100 to 200 loaves, and more than 200 loaves was respectively 90, 88, 194, and 259 μg/g.

Thus dose-effect relationships for methylmercury compounds have been indicated for dose estimated variously, e.g. as ingested amount, blood concentration, hair concentration, and brain concentration. Unfortunately, despite the wealth of data available on dose-effect and dose-response relationship for methylmercury compounds, the critical effect has to be defined as broadly as paresthesia. No other effect has yet been recognized at lower doses.

IV.5.4.4.1.3 Methylmercuric compounds: dose-response relationships (describing exposure-response)

Comparatively little information is available on the relationships between intake and response in humans. However, measurements of concentrations of total mercury or $MeHg^+$ in various indicator (or index) media in the outbreaks in Japan and Iraq and in populations having high dietary intake of fish have allowed an estimation of the dose, using a metabolic model of $MeHg^+$ in man.

The minimum concentrations in the brain (critical organ) associated with signs and symptoms have been reported in Table 10. The section on "critical concentration in critical organ" (section IV.5.4.4.1.1) discusses the sources of evidence for these minimum critical concentrations. The evidence on dose-response relationships comes from the same source. The concentration of mercury in indicator (or index) media (blood, hair) and the maximum body burden may be calculated from the metabolic model. For example, the most consistent figure for the minimum brain concentration associated with signs and symptoms, i.e. 1 µg/g, corresponds to a blood level of 200 ng per gram and a hair value of 60 µg/g. The calculated body burden for a 70 kg person would be about 20 mg corresponding to approximately the same amount of totally ingested $MeHg^+$ in an acute exposure.

Detailed dose-response relationships in which dose was estimated as body burden of $MeHg^+$ (corresponding to approximately the same acute exposure) are quoted in Figure 17. The general principles involved in the relationship have been discussed elsewhere (IV.5.1). The data indicate that considerable individual variability exists in human sensitivity to methylmercury compounds. The data in Figure 17 point to a factor of 10 or so as measured by the frequency of paresthesias. Variability is less with signs of poisoning such as ataxia and dysarthria.

Nordberg and Strangert (1975) have noted that, if dose is to be expressed as a daily intake, another important variable must be taken into account, i.e. the clearance half-time for man of which the wide individual variability has been indicated by studies on hair by Al-Shahristani and Shihab (1974). The range was from 40 to more than 120 days. In two individuals having the same daily intake of $MeHg^+$, but having clearance half-times of 120 versus 40 days, the first would accumulate a body burden three times higher than the second upon long-term exposure to $MeHg^+$. By taking into account the distribution of interindividual variation in critical concentration (as reported by Bakir et al., 1973; Figure 17) and the variation in biological half-time, Nordberg and Strangert calculated a relationship between daily dose and probability of poisoning at long-term exposure to methylmercuric compounds.

In the fetal cases of methylmercury poisoning reported by Amin-Zaki et al. (1974) (and discussed further by Clarkson and Marsh, 1975), it was found that of 8 infants born to mothers with blood $MeHg^+$ concentrations of 50 to 500 ng/ml only 2 were affected, whereas 3 out of 3 infants were affected when the maternal blood concentration was above 1,000 ng Hg/ml. Body burdens (= total short term exposure) corresponding to these blood concentrations are 5 to over 100 mg. A more precise dose-response evaluation cannot be made on basis of these limited observations.

IV.5.4.4.2 Ethylmercuric compounds

As was mentioned earlier, no adequate data are available to discuss dose-effect relationships in critical organs and indicator (or index) media, or critical concentrations in critical organs and indicator (or index) media.

IV.5.4.4.3 Other organomercury compounds

As said previously the critical organ and the critical effect for phenylmercury exposure are not easily definable, but the kidney is the probable critical organ and the critical effect most likely is changes in the renal tubules. A detailed evaluation of phenylmercury involving critical organ concentration and metabolic models is an even more difficult task. There are no conclusive data which permit definite assertions about mercury concentrations in the kidney at exposure to phenylmercury compounds. In animal experiments, levels of mercury in the kidney have been found close to those seen at poisoning due to mercuric salts, but differences in absorption between the two compounds on oral exposure make total mercury concentrations higher for phenylmercury than for mercuric salts. Still, concentrations which are connected with signs of toxicity seem to be the same. Fitzhugh et al. (1950) found a mean kidney level of 2.3 µg/g in rats showing slight morphological changes, and 39 µg/g in rats showing severe changes. Tryphonas and Nielsen (1970) reported between 160 and 370 µg/g in the kidneys of pigs with severe renal damage. A summary of available data has been given by Skerfving (1972), in which ingestion of 100 mg of mercury as a phenylmercuric compound was described as having caused only slight gastrointestinal symptoms. In another case, laboratory investigation and renal biopsies showed normal conditions after a person had ingested as much as 1,250 mg Hg. The oral toxicity of phenylmercuric compounds is thus obviously rather low.

There is evidence for absorption of mercury from phenylmercuric compounds applied onto the surface of the skin and into the vagina. Mercury concentrations in urine in connection with albuminuria have been 8.5 mg/l in one case; levels up to 6 mg Hg/l in spot samples of urine have been published for workers exposed to phenylmercuric salts and considered to be free of symptoms and signs.

Specific data about mercury concentrations in the kidney at exposure to methoxyethylmercury compounds are not available. Lehotzy and Bordas (1968) found histological changes similar to those found after intoxication with mercuric chloride in the kidney of rats exposed to methoxyethylmercury chloride intraperitoneally. These authors have also found some neurological effects after exposure to this type of mercurial, and it may therefore be questioned as to whether the kidney is really the critical organ. On the other hand, the possibility cannot be excluded that a primary kidney damage can also give rise to symptoms of a neurological character as a secondary manifestation of uremia.

IV.5.4.5 Factors affecting dose-response relationships for mercury and its compounds

Many factors (host selected, other chemicals) could influence the dose-response relationship. This text will be restricted to examples for which evidence in experimental animals or in man points to a reasonable probability of influencing the dose-response relationship. Four general factors will be discussed: (a) the development of tolerance, (b) sensitivity to mercury as related to the life cycle, (c) other sensitivities and idiosyncratic reactions, (d) interaction with other chemicals.

(a) Pretreatment with a small dose of cadmium or mercury protected against the subsequent toxic dose (Yoshikawa, 1970). Tolerance can also develop in the course of daily injection of 0.5 mg/kg $HgCl_2$ for 14 days: at the end of this period, virtually complete regeneration occurred and regeneration was advanced even in rats receiving 2.0 mg/kg $HgCl_2$ (Prescott and Ansari, 1969). Increased tolerance to cadmium may be due to metallothionein formation.

(b) Studies on rats reported by Greenwood et al. (1972) indicate transplacental passage to the fetus in exposures to elemental mercury vapor. However, no information is available on the effects of prenatal exposure to the vapor in humans.

Evidence of prenatal poisoning in the Minamata outbreak has been reviewed (Berglund et al., 1971). Twenty-two infants whose mothers had been exposed to methylmercury compounds were born suffering from cerebral palsy, but exhibited only slight effects of methylmercury poisoning. Early data on infant-mother pairs in whom prenatal exposure had occurred in the Iraqi outbreak have been quoted by Clarkson and Marsh (1975). No higher frequency of poisonings in the infants as compared to the mothers was detectable. However, only twelve infant-mother pairs were studied and the observations were made soon after the epidemics. A greatly expanded follow-up study is now in progress.

(c) Certain clinical manifestations such as hypersensitivity, idiosyncrasy and acrodynia (pink disease) can be caused by inorganic or organic forms of mercury (Clarkson, 1972a, 1972b).

(d) Interactions of other chemicals with mercury are under discussion in other sections (IV.6.3) of this report. It may be mentioned here that the concomitant exposure to ethanol decreases the pulmonary absorption and toxicity of mercury vapor. Animal experiments have shown that exposure to aminotriazole can modify distribution of mercury (section IV.3.3.3.4.1). Protection by selenium against the toxic effects of inorganic mercury has been demonstrated (section IV.6.2). Selenium protection has been witnessed only for extremely high dietary levels of

$MeHg^+$ (20 to 40 μg/g in the diet, Ganther et al., 1972). The relevance of the studies on animals to actual $MeHg^+$ levels in human diet (less than 1 μg/g) remains to be established. No information is available so far on selenium-$MeHg^+$ interaction in man.

IV.5.4.6 Summary of dose-response relationships for mercury and its compounds

This summary will comprise the major conclusions as to critical organ, critical concentration and critical effect, and the corresponding concentrations of mercury in indicator (or index) media. It should be borne in mind that considerable individual variability exists in susceptibility to mercury in all of its forms. In the case of methylmercury compounds, the variability in the human population has been indicated to be on the order of a factor of ten.

Considerable information is at our disposal on critical concentrations of methylmercury compounds. Still, there is a clear need to improve tests and diagnostic procedures in order to detect effects prior to the onset of paresthesia, which is the slightest effect known at present to be associated with exposure to methylmercuric compounds.

With respect to Hg^o, information on dose-response relationships is available for long-term exposure. Nothing is known about brain levels of mercury in humans at the time of onset of the signs and symptoms of poisoning. The critical organ is difficult to assign to a particular sign or symptom. The dose-response relationships reported in one industrial study indicate complaint of such symptoms as loss of appetite, insomnia, and shyness, and signs, e.g. tremor, as related to certain air levels of mercury.

The acute effects of inorganic mercury are well described. The concentrations in blood, hair and urine at the time of onset of kidney failure have not been reported. The consequences of long-term exposure to inorganic mercury in man are not known. Animal experiments indicate the kidney as the critical organ. Dose and concentration relationships cannot be specified in part because of lack of data and in part because of the possibility of the development of tolerance.

Information on the toxicology of organomercurials in man other than methylmercury compounds is meager. Based on their known rapid conversion to inorganic mercury in animals, it is possible that the effects are similar to those of inorganic mercury.

Factors affecting the dose-response relationship were discussed. Alcohol, aminotriazole, and probably other inhibitors of catalase markedly affect the metabolism of mercury vapor. The possible protection of humans by selenium against the toxicity of methylmercury compounds is a question of considerable importance with respect to risks for oceanic fish ingesting these substances.

IV.6 FACTORS INFLUENCING THE EFFECTS AND DOSE-RESPONSE RELATIONSHIPS FOR TOXIC METALS; THE EFFECTS OF SELENIUM AND METAL-METAL OR METAL-MINERAL INTERACTIONS

IV.6.1 Introduction

Definition: Interaction is a process by which metals in their various forms or other factors change the critical concentration or a critical effect of a metal under consideration. In this section the term interactions will also be used to describe the effect of certain elements on the absorption, excretion, retention, and toxicity of cadmium, mercury and lead.

Even though it has been shown that certain organic nutrients such as proteins (Fitzhugh and Meiller, 1941; Suzuki et al., 1969) and ascorbic acid (Evans, 1973; Fox, 1974), interact with cadmium, that dietary protein, vitamins D and C, and ethanol (Goyer and Mahaffey, 1972) interact with lead, and that vitamin C (Magos, 1973) and ethanol (Magos et al., 1973) interact with mercury, these types of interaction will not be discussed in this report. Furthermore, the following text will mainly rely on data from animal experiments, since there are very few data on metal interactions in human beings.

The following interactions will be considered:

(a) Interaction of selenium with mercury and cadmium

Because selenium is known to affect the toxicity of both these and some other metals in a similar way, and since this topic has been extensively investigated, the Subcommittee decided to deal with it separately.

(b) Some interactions of cadmium, mercury and lead with other metals or minerals

The molecular mechanisms underlying these interactions are not well understood. Various aspects of the hypotheses and theories of proposed mechanisms for these interactions can be found in this volume (Magos, 1975; Parizek, 1975; Sandstead, 1975).

IV.6.2 Interaction of selenium with cadmium and mercury compounds

Selenium has been shown to protect against several of the toxic effects of cadmium. This was first demonstrated for cadmium-induced testicular necrosis (Kar et al., 1960; Mason et al., 1964; Mason and Young, 1967; for review see Gunn and Gould, 1970; Friberg et al., 1971 or 1974). Since then selenium has been shown to protect against cadmium-induced hemorrhagic necrosis of nonovulating ovaries (Parizek et al., 1968a), necrosis of placenta (Parizek et al., 1968b), a toxemia-like syndrome in the third period of pregnancy in rats (Parizek et al., 1968b), teratogenic effects in hamsters (Holmberg and Ferm, 1969), lethal effects in non-pregnant rats (Parizek et al., 1968b) and in mice (Gunn et al., 1968) and elevation of blood pressure in rats (Perry and Erlanger, 1974).

Selenium protects against the lethal effect of inorganic mercury salts in rats; at the same time the rats are protected from renal tubular and intestinal necrosis (Parizek and Ostadalova, 1967; Parizek, 1971a). Selenite added to food will enhance the growth of rats receiving mercuric chloride by mouth (Potter and Matrone, 1974). Dietary selenium as sodium selenate partially protects from chronic nephritis induced by peroral mercuric chloride in rats, even though there is an increase in the mercury concentration in the kidney (Groth et al., 1973; Groth et al., 1975).

Toxic effects induced by methylmercuric salts are also prevented by inorganic selenium. Selenite was shown to prevent growth inhibition as well as the lethal and neurotoxic effects of methylmercury compounds when administered simultaneously in the diet of rats and Japanese quail (Ganther et al., 1972;

Stillings et al., 1972; Potter and Matrone, 1974; Ueda et al., 1974; Ohi et al., 1975). In chronic studies with dietary exposure to $MeHg^+$ and selenite, control rats exposed to $MeHg^+$ died with symptoms of neurotoxicity and concentrations of $MeHg^+$ in the brain well below those present in rats protected by selenite (Ueda et al., 1974; Ueda et al., 1975). Selenite given parenterally also decreased the toxicity of dietary $MeHg^+$ while at the same time temporarily increasing the $MeHg^+$ concentration in rat brain (Iwata et al., 1973).

Similar protective effects of selenium have been described for the toxic effects of thallium (Hollo and Zlatarov, 1960; Rusiecki and Brzezinski, 1966), silver (Diplock et al., 1967) and arsenic (Levander and Argrett, 1969).

At the same time that selenium protects the critical organs from the toxic effects of these metals, it can also increase their concentrations in critical organs. (Critical organs as discussed here sometimes refer to sites of effects seen after injection of relatively large single doses of metal salts, a situation not given much attention in previous chapters).. The best demonstration of this course of events is the ability of selenium to prevent the necrotizing effect of cadmium on the testicles, and at the same time to produce a several-fold increase in the concentration of cadmium in this organ (Gunn and Gould, 1970). A recent study indicates that this might be related to a concomitantly occurring change in the binding of cadmium to certain proteins in the testes (Chen et al., 1974). Under certain conditions the oral administration of selenium will increase the concentration of inorganic mercury in the kidneys without an apparent increase of the chronic toxic effect (Groth et al., 1975).

Selenium exposures that decrease cadmium or inorganic mercury toxicity have been shown to increase markedly the concentration of the respective metals in blood (Parizek et al., 1969a, 1969b, 1974). A recent paper has shown that mercury and selenium are bound to a plasma protein in an atomic ratio of 1:1 (Burk et al., 1974). A binding of this type may explain alterations in the biological activity of metals and in the biological availability of selenium when selenium and the metals interact in the organism, as referred to by Parizek (1975) and in a recent review (Parizek et al., 1974). This could also explain the altered distribution of the metals, as well as the decreased passage of mercury and selenium across the placenta and into the milk when inorganic salts of these elements are given simultaneously (Parizek et al., 1969a, 1969c, 1971a, 1971b). It remains to be shown if selenium can affect the transplacental passage of $MeHg^+$ and its subsequent fetal effect.

The report that $MeHg^+$ in tunafish and large oceanic fish is less toxic than $MeHg^+$ given under other circumstances, and the hypothesis that selenium could be one of the factors involved (Ganther et al., 1972; Ueda et al., 1974) deserve to be considered as issues of moment. High concentrations of mercury and selenium have been found in livers and brains of apparently healthy sea mammals. The atomic ratio of mercury to selenium in these cases was again approximately 1:1 (Koeman et al., 1972, 1973).

An interrelation for the better between mercury and selenium has also been reported recently in human subjects exposed to inorganic mercury (Kosta et al., 1974). Inorganic compounds of mercury and arsenic, in contrast, increase the toxicity of dimethylselenide or trimethyl selenium salts by several orders of magnitude (Parizek et al., 1971a, 1974; Palmer and Halverson, 1974). Further research is needed to show whether or not $MeHg^+$ is capable of producing this effect. These examples illustrate how interactions of a toxic metal with selenium can have adverse effects, as well as beneficial effects, on the dose-response relationship.

IV.6.3 Mercury

Interactions of mercury with other minerals have received little attention, with the exception of its interactions with selenium discussed above. Cadmium pretreatment has been shown to influence the distribution of mercury, resulting in higher kidney levels of mercury in rats (Magos et al., 1974x). In the same groups, cadmium protected the kidneys from the renotoxic effects of mercury. Long-term exposure to $HgCl_2$ may bring about some tolerance (Prescott and Ansari, 1969). The mechanism for these effects might involve the binding of mercury to metallothionein.

IV.6.4 Cadmium

One of the first interactions discovered for cadmium was the protective effect of zinc against testicular necrosis induced by injection of cadmium (Parizek, 1957). The total dose of zinc needed to offer complete protection against cadmium amounted to about 100 times the molar equivalent of cadmium (Parizek, 1956, 1957, 1960; Gunn et al., 1961). The protective effect of zinc is believed to be partly dependent on the induction of an increased synthesis of metallothionein-like proteins[x] (Webb, 1972b; Davies et al., 1973). There is some uncertainty as to whether zinc actually induces synthesis of metallothionein (Shaikh and Lucis, 1970; Friberg et al., 1974). As pointed out previously (sections IV.4.2.1 and IV.5.2.4), both cadmium and zinc bind to metallothionein and induction of the synthesis of this protein therefore is likely to influence the turnover and toxicity of these two metals. It may be of interest to mention in this context that Cotzias et al. (1961, 1962) reported that administration of zinc salts increased the cadmium retention in the body.

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[x] The term "metallothionein-like proteins" is used to indicate that the proteins had some characteristics in common with metallothionein (e.g. molecular size) but that a complete identification has not been reported.

The mechanism involving metallothionein seems to explain some but not all of the observations on zinc-cadmium interactions. The possibility that the protective effect of pretreatment by zinc on testicular cadmium necrosis is related to the induction of metallothionein corresponds well with the time interval necessary between the administration of zinc and cadmium for development of resistance to cadmium (Webb, 1972; Chen et al., 1974). On the other hand this mechanism alone does not explain why simultaneous administration of zinc with cadmium completely protects against cadmium-induced testicular necrosis (Mason and Young, 1967). These latter observations seem to indicate that a different interaction could be operating, involving competition for ligands involved in the transport of cadmium, or involving metal binding ligands in the target tissue itself.

The teratogenic effect of cadmium is prevented by the simultaneous injection of zinc (Ferm and Carpenter, 1968).

The interaction between zinc and cadmium appears to be important for human health. The levels of cadmium and zinc in kidneys from human beings without known industrial exposure have been measured in autopsy material (see section IV.5.2.4). With age, the levels of cadmium and zinc increase in parallel and in equimolar amounts in normal people (Piscator and Lind, 1972). In hypertensive patients, an increase in the ratio of cadmium to zinc has been observed (Schroeder, 1967; Lener and Bibr, 1971).

Experiments in the rat have shown that cadmium will induce increased blood pressure under carefully controlled conditions (Schroeder and Vinton, 1962; Perry and Erlanger, 1974). These studies have also shown that the levels of cadmium required for such an effect are relatively low, and that when high doses are given, increased blood pressure does not occur (Perry and Erlanger, 1974). In addition, it appears that the level of zinc in the diet is also quite specific (Perry, 1974; Doyle et al., 1975). When rats were given 2.5 μg of Cd/ml plus 12.5 μg of zinc/ml in the drinking water along with 23 μg of zinc/g of the diet, their blood pressures were not significantly greater than those of the controls (Perry, 1974; Sandstead, 1975). When the zinc intake was increased to 50 μg/ml with 2.5 μg Cd/ml of water and the dietary zinc concentrations were unchanged, the animals developed hypertension. At a higher level, 100 μg/ml of water, zinc was antagonistic to cadmium and increased blood pressure did not occur. Thus, zinc was antagonistic to cadmium at some levels and "synergistic" with cadmium at others.

Analysis of renal cortex from rats has shown that the ratio of cadmium to zinc in the tissue is better correlated to the occurrence of hypertension than to the absolute level of cadmium. Thus, animals with a molar ratio of cadmium to zinc of less than 0.37 were not hypertensive and rats with a ratio of 0.46 were always hypertensive (Schroeder et al., 1966). Additional evidence for the idea that cadmium is an etiological factor in hypertension is the finding that its removal from the above hypertensive rats by a zinc chelate resulted in a cure of the hypertension (Schroeder, 1967). While the mechanism of the toxicity of cadmium on the kidney has not been defined, one effect which may relate to the induction of hypertension is the sodium retention which is caused by cadmium (Lener and Musil, 1971; Doyle et al., 1975. Presumably the sodium retention is related to an increased level of renin secretion by the kidney (Perry and Erlanger, 1971). In addition to these studies in which hypertension has been produced, there are also negative reports in this respect. Part of the explanation may lie in the genetic variability in susceptibility (review: Friberg et al., 1974). The findings of hypertension in the rat are not in agreement with observations on industrial workers exposed to cadmium. Perhaps the disparity is related to the levels of cadmium exposure as well as to the levels of zinc and/or selenium in the diets of the subjects.

Studies in man which may relate to the observations in the rat are those which suggest that the cadmium content of drinking water (soft water) may be a factor in the occurrence of hypertensive cardiovascular disease. This hypothesis has not been adequately tested. At present it can only be stated with confidence that the softness or acidity of water correlates with the incidence of cardiovascular disease. Such water is more likely to have a relatively high concentration of cadmium than hard water (Perry, 1973).

An interaction between cadmium and copper has been shown in the intestinal mucosa (Evans and Hahn, 1974; Hahn and Evans, 1974). Both cadmium and copper complex with a metallothionein-like protein present in the intestinal epithelial cells. It is not known whether this protein is identical with metallothionein or with a slightly different copper binding protein with a molecular weight almost identical with metallothionein and recently isolated from the liver (Winge et al., 1974). The intestinal protein has also been found within the intestinal lumen (Evans et al., 1974). It appears to facilitate the intestinal absorption of copper and to have a strong influence on the intracellular metabolism of copper (Evans, 1973). It is unknown whether the binding of cadmium to this protein in some way impairs the metabolism of copper in the intestine.

A nutritional interaction between calcium and cadmium has been observed (Larsson and Piscator, 1971; Piscator and Larsson, 1972). Low levels of dietary calcium increase the absorption of cadmium by the rat. Conversely, it is probable that cadmium can interfere with calcium absorption through its toxic effect on the renal tubules and inhibition of the formation of the vitamin D metabolite 1-25$(OH)_2$ cholecalciferol (Feldman and Cousins, 1973; Kimura et al., 1974). Calcium may also influence the toxicity of cadmium through its presence in hard water (see above).

Altered sensitivity can be achieved by pretreating the animal with zinc, selenium and certain other metals. In addition, pretreatment with small doses of cadmium will protect from a subsequent injection of a larger dose of cadmium (Terhaar, 1965; Ito and Sawauchi, 1966; Gabbiani et al., 1967b; Yoshikawa, 1970, 1973; Nordberg, 1971, 1972a). While the mechanism of this phenomenon has not been established, it seems probable that the induction of metallothionein and subsequent sequestration of cadmium are at least partly responsible (Nordberg, 1971, 1972a).

IV.6.5 Lead

The most studied interactions of lead with essential metals are those with iron and calcium. Lead probably interferes with iron metabolism at two levels. It blocks the introduction of iron into the tetrapyrole ring by inhibiting heme-ferrochelatase activity (Chisolm, 1964). Bessis and Jensen (1965) have shown that iron in the form of apoferritin and ferruginous micelles accumulates in mitochondria of bone marrow reticulocytes in lead-poisoned rats.

There may also be some relationship between iron and lead absorption in the gastrointestinal tract. Iron deficient rats show an increased absorption of dietary lead (Six and Goyer, 1972). This finding may be particularly significant in terms of childhood lead effects, since many young children with elevated blood lead levels also have iron deficiency.

The interaction between calcium and lead has been shown most dramatically in the rat. Rats fed low calcium diets took up sufficient ambient lead to show an increase in blood lead and urinary ALA excretion compared to animals given adequate dietary calcium (Mahaffey and Goyer, 1973). Also, the lead content of soft tissues as well as biochemical and morphological manifestations of lead toxicity were more severe in rats fed low-calcium diets than in rats fed normal dietary calcium, both groups having received lead in drinking water. Kostial et al. (1971) found that retention of lead by weanling rats is decreased when calcium and phosphate are added to milk.

There may also be a relationship between zinc and lead metabolism, although such studies are relatively incomplete. The feeding of toxic amounts of zinc to horses prevents manifestations of lead toxicity (Willoughby et al., 1973). At the cellular level, it has recently been shown that the fluorescent porphyrin, or so-called FEP, elevated in patients with lead poisoning, may indeed not be free but instead chelated with zinc. Zinc protoporphyrin is also increased in erythrocytes of children with iron deficiency anemia (Lamola and Yamane, 1974). The influences of these relationships upon manifestations of lead poisoning or blood lead levels are yet unknown.

IV.6.6 Conclusions

Though research on interactions affecting the metabolism and action of those metals discussed in the present report (i.e. cadmium, mercury, lead) is still undergoing a rapid development, certain conclusions can already be drawn which are pertinent to the effects of these metals and their respective dose-response relationships.

Of particular interest are those interactions which increase the body burden of the toxic metal, its concentration in the critical organ, and/or in media used for quantitation of its presence, and which at the same time influence the toxic effects. This has been demonstrated in some of the experiments noted above. Studies of this type have identified some of the factors which should be included in the protocol of studies on the relation between the dose and the effect of toxic metals. This conclusion pertains both to epidemiological studies and prospective experimental work.

In addition, a better understanding of interactions may open new possibilities for the prevention and/or therapy of adverse effects. From the point of view of public health, it is of particular importance to study the interaction between two chemicals (or metals) when there is a high probability that populations will be simultaneously exposed to both.

IV.7 ACKNOWLEDGMENTS AND LISTS OF WORKING PAPERS AND PARTICIPANTS

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During the meeting in Tokyo the administrative work was supervised by Dr. Y. Seki and Ms. Cecilia Hamagami. Ms. Birgitta Morin and Ms. Elisabeth Mueller supervised the secretarial work.

The Editor, Editorial Committee, and the Administrative Editorial Assistant met at the Environmental Protection Agency, Research Triangle Park, N.C., USA, for final decisions on editorial matters. The editorial work was performed in part while Dr. Nordberg was a visiting professor at the Department of Pathology, University of North Carolina, Chapel Hill, N.C., USA under a grant from US Environmental Protection Agency.

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LIST OF WORKING PAPERS NOT INCLUDED IN THIS VOLUME

The following reports were presented and treated as working papers at the meeting but have been or will be published elsewhere. They will be listed here in full.

Goyer, R.A. and Rhyne, B. (1973). Pathological effects of lead. In: "International Review of Experimental Pathology." (G.W. Richter and M.A. Epstein, eds) Vol. 1 pp 1-77, Academic Press, New York.

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